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of  
Congenital Cytomegalovirus  
Infection**

by  
**Karin Ahlfors**



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EPIDEMIOLOGICAL STUDIES OF CONGENITAL CYTOMEGALOVIRUS INFECTION

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| <p><b>Abstract</b></p> <p>Prospective virological and clinical studies of congenital cytomegalovirus (CMV) infection were performed on 8,367 Swedish infants. In half of the material, all mothers were serologically investigated: 72% had antibodies to CMV at their first antenatal visit; seroconversion (primary infection) was demonstrated in 1.2% of the seronegatives; 43% of the primary infections resulted in congenital infection. Of all infants congenitally infected, two thirds were born to mothers with serological patterns compatible with secondary infection. Congenital CMV infection, shown by virus isolation within the first week of life, was found in 0.5% of the infants studied. In 20%, (usually mild) symptoms referable to the reticuloendothelial organs occurred. After a follow-up ranging from six months to four years, 29% of the neonatally symptomatic infants and 8% of the asymptomatic ones or totally 12% had neurological sequelae. This is equivalent to 0.06% in the general population. Both primary and secondary maternal infections were shown to cause disease in the infants but, as illustrated in a separate study including severely affected infants found in routine work, the major threat to the offspring seemed to be primary maternal infections in first and second trimesters. Attempts to demonstrate pregnancies at risk would have been successful in only half of the cases of infant sequelae.</p> <p>The risk of CMV infection in nurses and their offspring was studied in 292 women working in paediatric clinics or day nurseries. In addition, the occupation of 36 mothers of infants with congenital CMV infection was investigated. There was little or no evidence of increased risk in the two materials.</p> <p>The immunity status to CMV was studied in 1,422 individuals belonging to a normal urban Swedish population. In the age group 1-4 years, 40% were seropositive. At 15-20 years of age this frequency started to increase and reached 85-95% at ages above 40.</p> |                                           |          |  |  |
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EPIDEMIOLOGICAL STUDIES OF CONGENITAL CYTOMEGALOVIRUS INFECTION

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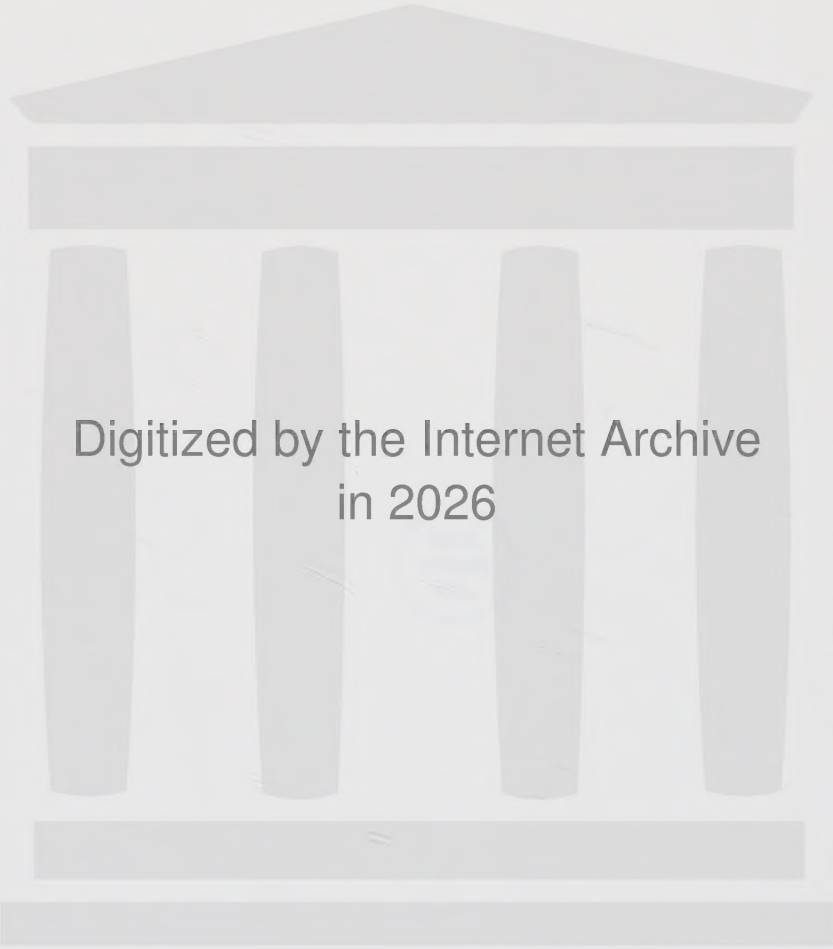
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## CONTENTS

|                                                              |    |
|--------------------------------------------------------------|----|
| Abbreviations .....                                          | 6  |
| List of papers .....                                         | 7  |
| Introduction .....                                           | 8  |
| Object of the study .....                                    | 8  |
| General description of CMV, CMV-infection and -disease ..... | 8  |
| Previous personal studies not included in the thesis .....   | 11 |
| Purpose of the present investigation .....                   | 13 |
| Methods .....                                                | 13 |
| Synopses of papers I - V .....                               | 16 |
| Discussion .....                                             | 22 |
| Conclusions .....                                            | 29 |
| References .....                                             | 30 |
| Acknowledgements .....                                       | 36 |

## ABBREVIATIONS

|       |                                             |
|-------|---------------------------------------------|
| AGA   | = appropriate for gestational age           |
| APR   | = aureo-palpebral reflex                    |
| BOEL  | = a type of contact test named after a girl |
| CF    | = complement fixation                       |
| CID   | = cytomegalic inclusion disease             |
| CMV   | = cytomegalovirus                           |
| CNS   | = central nervous system                    |
| CPE   | = cytopathic effect                         |
| EBV   | = Epstein-Barr virus                        |
| ECoG  | = electrocochleographi                      |
| ELISA | = enzyme-linked immunosorbent assay         |
| ELA   | = enzyme-labelled antigen                   |
| HEL   | = human embryonal lung                      |
| HSV   | = herpes simplex virus                      |
| IgG   | = immunoglobulin G                          |
| IgM   | = immunoglobulin M                          |
| IIF   | = indirect immunofluorescence               |
| RF    | = rheumatoid factors                        |
| SGA   | = small for gestational age                 |
| VZV   | = varicella-zoster virus                    |

## LIST OF PAPERS

This thesis is based on the following papers referred to in the text by their Roman numerals:

- I Ahlfors, K., Ivarsson, S.-A., Johnsson, T. & Svanberg, L.: Primary and secondary maternal cytomegalovirus infections and their relation to congenital infection. Analysis of maternal sera. Acta Paediatr Scand 71:109, 1982.
- II Ahlfors, K., Ivarsson, S., Harris, S., Svanberg, L., Holmqvist, R., Lernmark, B. & Theander, G.: Frequencies of congenital cytomegalovirus infection and disease in Sweden and relative significance of primary and secondary maternal infections. A prospective virus isolation study. Accepted by Scand J Infect Dis 1982.
- III Ahlfors, K., Forsgren, M., Ivarsson, S., Harris, S. & Svanberg, L.: Congenital cytomegalovirus infection. On the relation between type and time of maternal infection and infant's symptoms. Accepted by Scand J Infect Dis 1982.
- IV Ahlfors, K., Ivarsson, S.-A., Johnsson, T. & Renmarker, K.: Risk of cytomegalovirus infection in nurses and congenital infection in their offspring. Acta Paediatr Scand 70:819, 1981.
- V Ahlfors, K.: Immunity to cytomegalovirus in a normal Swedish population. Accepted by Scand J Infect Dis 1982.



## INTRODUCTION

### History

Inclusion-bearing cells characteristic of cytomegalovirus (CMV) in the kidneys of a stillborn infant who died of syphilis were observed by Ribbert in Germany as early as 1881 (51). Similarities of the inclusions in varicella and herpes simplex were reported in 1921 (23,44). Later, Farber et al coined the term "salivary gland virus disease" (19). Experimental evidence corroborating the viral etiology of the disease was produced in 1926 by Cole et al (13). Human virus was isolated independently by Smith (58), Rowe et al (52) and Weller et al (69) in 1956 and 1957. In 1960 Weller et al (70) proposed the term "cytomegalovirus" for the virus. In Sweden, virological studies of CMV were first performed by Carlström (11).

### OBJECT OF THE STUDY

Besides being an important pathogen in adults, CMV is the most common cause of congenital viral infection in man (63). Accordingly, there has been good reason to focus interest on the diagnostic and clinical problems of this subject. Below-mentioned data of a generally accepted character have either been obtained from reviews by Hanshaw (30) or Hanshaw and Dudgeon (31) or textbooks of general virology.

### GENERAL DESCRIPTION OF CMV, CMV-INFECTION AND -DISEASE

Virus description. CMV is an enveloped icosahedric virus with linear double-stranded DNA. It is regarded as a separate genus within the Herpesviridae family. The genus also includes several CMV strains of non-human origin. CMV is one of four herpes viruses in man. The others are Herpesvirus hominis (herpes simplex) (HSV), Herpesvirus varicellae (varicella-zoster) (VZV) and Epstein-Barr virus (EBV). Infections with HSV or VZV are generally not considered to elicit heterotypic antibody rise to CMV (6,37,1). In EBV infection, IgM response to CMV has been observed (20,29). Antigenic diversity among CMVs is so considerable that repeated efforts to sort them into serotypic groups have been futile (50). No two epidemiologically unrelated isolates have identical DNA fragment patterns; however, the results obtained to date indicate that human CMV strains share at least 80 % of their genetic information (35). Some prototypic strains do exist such as the Davis, AD-169, Kerr, Esp and Towne-125 strains.

Characteristic cytopathology consists of greatly enlarged cells containing both intranuclear and cytoplasmatic inclusions. Another characteristic feature of the CMV-infected cell is its Fc-IgG-binding capacity (36). Although human CMV in vivo primarily involves epithelial cells, human fibroblastic cells are necessary for in vitro growth.

Excretion; occurrence. Excretion occurs with urine, saliva, semen, tears, milk, stools, and vaginal and cervical secretions. CMV can also be isolated from plasma and blood elements, as well as from tissues obtained by biopsy or autopsy. A unique feature of CMV is the long persistence of viral excretion: children shed virus for years, adults for months after primary infection.

Transmission. Transmission is mediated by close interpersonal contact or direct transfer of infected cells or fluids.

Incubation period. From studies in patients receiving transfusions (42) and infants with perinatally acquired infection (43), the incubation period seems to be about 1 month with variations from a few weeks to a few months.

Types of infection. Characteristic of the Herpesviridae family is the latent infection following the primary one. Reactivation is common. Based on DNA pattern analyses the majority of the recurrent CMV infections represent reactivation of existing latent virus; however, reinfections by a new virus strain do occur occasionally (35). We have used the joint term secondary infection for reactivation and reinfection.

Clinical manifestations. Most CMV infections in otherwise healthy individuals are subclinical. This is true for pregnant women and congenitally infected infants at the time of birth as well as for postnatally infected infants. Some of the congenitally infected infants have, however, symptoms. Most common are signs from reticuloendothelial organs (hepatomegaly, splenomegaly, thrombocytopenic purpura, icterus), but most serious is engagement of central nervous system (CNS) (microcephaly, intracerebral calcifications, chorioretinitis, optic atrophy). Such symptoms as pneumonitis, inguinal hernias and brachial arch abnormalities have occasionally been reported. Neurologic symptoms may also appear after the perinatal period.

The postnatally acquired infections in infancy might cause hepatosplenomegaly and pneumonitis (8,62). As distinguished from the congenital infection,

neurological sequelae have hitherto, with exception for one report, not been associated to the postnatally acquired type. Granström (24) reported infected infants to have retarded speech and possibly also retarded fine motor development.

In adulthood, acquisition of CMV might result in a mononucleosis-like disease (38).

In immunosuppressed individuals, CMV is an extremely common opportunistic virus. Both primary and secondary infections occur. In renal transplant recipients, fever, leucopenia and renal dysfunction dominate, while in bone marrow transplant recipients, the major impact of CMV appears to be in the pathogenesis of interstitial pneumonia. Recipients of cardiac transplants risk developing a syndrome characterized by fever, atypical lymphocytosis and pneumonia. Among long-term survivors of cardiac transplantation, progressive CMV retinitis has become an increasingly prevalent problem (53). Patients with leukemia might have pneumonitis and fever with rash attributable to CMV (34).

Allogenic stimulation may activate a CMV infection in latent state (49).

CMV has also been attributed to conditions such as Guillain-Barré syndrome (16) and haemolytic anaemia (12). It has been implicated as a possible oncogenic agent by virtue of its ability to transform hamster embryonic cells (3) and human fibroblasts (22). An increasing interest is paid to the role of CMV in Kaposi's sarcoma (17).

## PREVIOUS PERSONAL STUDIES NOT INCLUDED IN THE THESIS

The first isolations of CMV made by the author were accidental: some infants with symptoms of failure-to-thrive were found to excrete the virus. There was uncertainty regarding the clinical significance of these findings. A study of CMV infections in infancy was, therefore, started. The study resulted in two publications, the contents of which are presented below (1,2).

## CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTION. Virological and clinical studies on a Swedish infant population. (1)

The study included 2 clinical materials. Firstly, the frequency of CMV and its clinical significance were studied in 661 infants under 1 year of age admitted to the Paediatric Clinic in Malmö. Before the age of 1 week 4 (1 %) of 326 infants were shown to excrete the virus. At 1 month of age the frequency had risen to 6 (12 %) of 52 and thereafter it was some 20 - 25 %. Of infants born to immigrants, 60 % were infected at the age of >1 month. One of the 4 congenitally infected infants with perinatal CMV excretion had symptoms at birth and neurological sequelae at follow-up. The majority of the infections detected after the perinatal period seemed to be postnatally acquired and subclinical.

Secondly, an inventory was made of data on 18,695 infants born during a 6-year period. Two cases of virologically confirmed congenital CMV disease and 7 cases of microcephaly were found to be registered. Congenital CMV infection could be excluded in 4 of the 7 cases of microcephaly. The frequency of microcephaly caused by CMV should, accordingly, be  $\leq 3/18,000$  infants.

## SEROLOGICAL DIFFERENTIATION OF CONGENITAL AND ACQUIRED CYTOMEGALOVIRUS INFECTIONS DETECTED IN INFANCY. (2)

A serologic investigation was performed on 50 of the CMV-infected infants diagnosed in the previous study (1). The purpose was to differentiate, if possible, between congenitally and postnatally acquired CMV infections. Generally, the patients were studied by the indirect immunofluorescence (IIF) test for CMV-IgM antibodies in cord serum and by the complement fixation (CF) test for CMV antibody development.

Two of the 4 patients with virologically confirmed congenital infection had positive CMV-IgM tests in cord serum and 1 of the 2 had also a persistently high CF titer from birth to about 3 months of age. At this time, the amount of passively transferred maternal antibodies should have decreased signific-



antly and a persistently high titer should, accordingly, be a sign of early infant antibody production indicating congenital infection (60). In the remaining 46 cases, all with the CMV-excretion detected after 3 weeks of age, no positive IgM-reaction in cord serum or persistently high CF titer (13 cases studied) were recorded. Six of the 13 infants had a significant CF-titer rise after the age of about 3 months characteristic of postnatally acquired infection (60). However, one of the congenitally infected infants also had a similar antibody development.

It was concluded that the possibility of making a serological distinction of pre- and postnatally acquired CMV infections in a material of the above-mentioned type is limited because of missing adequate sera or uncharacteristic serological patterns.

#### Conclusions and practical consequences of the above-mentioned studies (1,2).

The essential result of the above-mentioned studies was the awareness of CMV as a common cause of infection in infancy and congenital CMV infection as a diagnostic problem. Only prospective studies performed on both mothers and infants would give reliable answers to questions regarding the frequency and symptoms of congenital infection and the relation between the maternal type and time of infection and the outcome of the offspring.

Malmö was considered to offer good conditions for prospective studies of the above-mentioned questions. For a number of years, sera from pregnant women and infected patients had been saved at the Obstetric Clinic and the Virus Laboratory. Such sera would be valuable in retrospective studies of the pre-conceptional immunological status of women becoming pregnant in the future. Most pregnant women in the town were examined at one antenatal clinic and all infants were born in one clinic which made tracing easier. Clinics and laboratories were localized close to one another permitting good communication. A prospective study based on a collaboration between the Obstetric, Paediatric and Virologic Departments at the Malmö General Hospital was started in the autumn of 1977. Later the work was extended to include active contribution or data from the Audiologic, Ophthalmologic, Psychiatric, Personnel Health Care, Roentgenological and Pathological Departments. A rewarding collaboration was also started with Dr Marianne Forsgren at the Virus Department of the Central Microbiological Laboratory of Stockholm County Council, Stockholm. The prospective study still continues, but sampling of material for the present thesis ceased in the autumn of 1981.

#### PURPOSE OF THE PRESENT INVESTIGATION

1. To assess the frequencies of congenital CMV infection and disease in a Swedish population and the type of symptoms in congenitally infected infants.
2. To determine the humoral immunity to CMV and the frequency of CMV-seroconversions in pregnant women and the frequency of primary maternal CMV infections transmitted to the fetuses.
3. To study the influence of primary/secondary maternal infections at different stages of pregnancy on the infants' congenital infection/disease.
4. To evaluate the possibility of tracing infants at risk for congenital CMV infection/disease by screening maternal sera.
5. To form an opinion of the risks of CMV infection in nurses and congenital infection in their offspring.

#### METHODS

Virus isolation. Urine samples were transported in an equal or smaller volume of transport medium consisting of Eagle's MEM with 1 % bovine serum albumin and antibiotics (penicillin 250 U/ml, streptomycin 50 µg/ml, neomycin 50 µg/ml and amphotericin B 2 µg/ml). At arrival to the laboratory the urine samples were enriched with twice the concentration of penicillin, streptomycin and neomycin and 10 times the concentration of amphotericin B. The fresh antibiotics were permitted to work for a short while before inoculation of the samples into tissue culture tubes.

Local strains of human embryonal lung (HEL) fibroblasts were grown in Eagle's MEM with 10 % newborn calf serum, penicillin (250 U/ml) and streptomycin (50 µg/ml). The composition of maintenance medium was the same except that it had only 3 % calf serum. The cells were shown to become more resistant to the toxic influence of urine if kept in the maintenance medium for a few days before use.

In the first 2 years, each urine sample was inoculated into 2 tissue culture tubes, both containing the same cell strain. In the next 2 years, the 2 tubes contained different cell strains, one type in each tube. With the latter arrangement, there was no apparent gain in the number of positive isolates provided both cell strains were in good condition, but it was a precaution against technical mishaps with one or the other of the tissue culture batches.

A volume of 0.2 ml, corresponding to about 0.1 ml urine, was inoculated into the medium (1.5 ml) in the tissue culture tubes. Through the entire 4-year period, except for 9 months in the last 2 years, separate urine samples were used as inoculate. During the 9 months, pools of 5 urine samples were used. Since the inoculated volume remained unchanged, each urine sample in the pools constituted about 0.02 ml. Because of some difficulties in reisolating and identifying positive pool findings in the separate samples (stored at +4°C), the pooling technique was subsequently abandoned. The tissue cultures were inspected weekly in a 4-week period.

During the whole study period the virus was identified by its typical CPE and its failure to replicate in epithelial cells. During the first 2 years, further identification was made by immunofluorescence using acute and convalescent human sera as indicators.

For virus titration fresh urine in transport medium was serially diluted 1/10 in phosphate buffer. From each dilutant 0.2 ml, corresponding to about 0.1 ml urine in the first step, was inoculated into each of 2 tissue culture tubes. Titers were expressed as TCID<sub>50</sub> of virus per 1 ml urine.

Serologic tests. The studies were based on 3 main serologic tests for demonstrating CMV antibody activity: the complement fixation (CF) test for combined IgG and IgM activities (10), the enzyme-linked immunosorbent assay (ELISA) and the indirect immunofluorescence (IIF) test for separate IgG and IgM activities, respectively. Complementary, IgM activity was in a few cases (in Stockholm) demonstrated with the ELISA and the enzyme-labelled antigen (ELA) techniques (54). Rheumatoid factors (RF) were demonstrated with Latex-RF-Reagenz (Behringwerke, West Germany).

The CF test was performed according to a micromethod described by Sever (56). The antigen was prepared by extraction of CMV-AD-169 infected HEL cells with alkaline glycine buffer (14). Control antigen was prepared in the same way from uninfected cells. Two units of antigen, 2 units of complement and a 0.25 % suspension of sheep's red blood corpuscles sensitised with 4 units of amboceptor were used. The serum dilution giving 50 % haemolysis was considered as positive end point, titers <8 as negative.

The ELISA-IgG was thoroughly tested in Paper I. It was a modification (68) of the micromethod originally described by Voller et al (71). The virus and the control antigens were similar to those used in the CF. Sera were tested at a dilution of 1/40 and antibodies indicated by an alkaline phosphatase conjugated swine anti-human immunoglobulin G (γ-chain specific) (Orion

Diagnostica, Finland). The absorbance of samples, expressed as  $E_{405}$ , was automatically recorded relative to that of control samples containing substrate, buffer and NaOH. The antibody content (E-value) was expressed as the  $E_{405}$  difference in tests performed with serum against virus and control antigens. E-values of  $>0.20$  were considered as positive.

The IIF-IgM test was a modification of a technique originally described for CMV by Hanshaw et al (28). Results from studies of control materials were reported in Paper III. CMV-AD-169 infected HEL cells grown on slides and fixed in  $+4^{\circ}\text{C}$  acetone were used as antigen. Sera were tested at dilutions of 1/16 and 1/64. Sera positive in the test were investigated for rheumatoid factors (57). Independent of the result of the RF test, a corroborative IgM test was (with few exceptions) performed after absorption of possible RF with aggregated human IgG (39). Consideration was given only to fluorescence of intranuclear inclusions. Titer 64 was considered as positive borderline value indicating an active, probably primary, CMV infection within the last 1 - 6 months. Also a significant ( $\geq$  four-fold) IgM titer rise below the level of 64 was considered as a sign of active infection (III).

Definition of congenital infection. This diagnosis was based on the demonstration of CMV excretion before the age of 1 - 2 weeks or on CMV isolation from fetal organs.

Exclusion of other congenital infections. In cases of congenital CMV infection complicated by neurologic sequelae the possibility of a coexisting rubella, toxoplasma or luetic infection was excluded by serologic tests.

Classification of maternal infection. Seroconversion was considered a sign of primary infection, but a high CMV-IIF-IgM level ( $\geq 64$ ) in the first post-conceptional serum obtained was also accepted. The diagnosis of (verified) secondary infection was based on the finding of preconceptional antibodies to CMV. Titer rise following a combination of positive CMV-IgG and negative CMV-IgM tests at the first antenatal control was considered suggestive of secondary infection. The antibody pattern most difficult to interpret was the combination of stable positive CMV-IgG and negative ( $<16$ ) CMV-IIF-IgM titers from the first antenatal visit until delivery. If the first serum was obtained in trimester I or early trimester II the pattern was considered likely to represent secondary infections (see discussion).

PRIMARY AND SECONDARY MATERNAL CYTOMEGALOVIRUS INFECTIONS AND THEIR RELATION TO CONGENITAL INFECTION. Analysis of maternal sera. (I)

Materials and methods. 4,382 new mothers were examined retrospectively with the ELISA for IgG activity to CMV during pregnancy. A comparison was performed between the ELISA and the CF test. Some of the mothers were also studied with the IIF test for CMV-IgM antibodies. Sera were regularly obtained at the first antenatal control and at delivery. As a rule, a number of intermediate sera were also obtained. In some cases of congenital infection in the infant, preconceptional sera were also studied. All the infants had been studied for CMV excretion within the first week of life (congenital infection).

Results. The ELISA-IgG proved to be a technically simple method with results similar to those obtained with the CF test.

Of 4,382 maternal sera obtained at the first antenatal visit, 1,218 (28 %) lacked IgG activity to CMV antigen. The percentage of seropositive women was 71 - 73 between 15 and 34 years; after that there was a slight increase. Samples obtained at delivery from 1,175 previously seronegative women showed ELISA-IgG seroconversion (primary infection) in 14 (1.2 %) cases. By studying intermediate sera, the approximate time of seroconversion could be dated (see below). Thirteen of the 14 CMV-IgG converters had a significant titer rise in the IIF-IgM test which in 12 cases showed a titer of  $\geq 64$ . In the IgM negative (IIF-titer  $< 16$ ) case, the sera showing IgG conversion were obtained with 3 months' interval. In none of the 14 cases was CMV infection suspected clinically.

Six (43 %) of the 14 women with primary infection gave birth to infants congenitally infected with CMV. The seroconversion of these 6 women occurred in pregnancy months III-X, IV-VI, V-IX, VI-IX, VI-IX and VII-VIII, respectively. The corresponding times for the mothers with uninfected infants were months III-IX, III-X, IV-IX, VI-VIII, VI-IX, IX-X, IX-X and IX-X, respectively.

Thirteen other infants found to be congenitally infected were born to mothers with CMV-IgG activity in their first antenatal serum sample. The maternal CMV infection was invariably subclinical. Twelve of the 13 mothers had a stable, positive CMV-IgG titer between the first antenatal check-up (usually in



months II-IV) and parturition. The 13th woman had a significant increase in her positive IgG titer between months IV and VI without significant CMV-IgM activity (the serological pattern suggestive of secondary CMV infection in trimester II). One of the 12 mothers with a constant IgG had a high IgM titer of 320 in month III interpreted as sign of primary infection in trimester I. The diagnosis was supported by negative serological tests 1 year before conception. In 4 cases preconceptional sera contained CMV antibodies (secondary infection). In the remaining 7 cases there was no serological indication as to the mothers' type of infection but for reasons mentioned in the discussion it was considered that most of these infections in reality were of secondary type.

FREQUENCIES OF CONGENITAL CYTOMEGALOVIRUS INFECTION AND DISEASE IN SWEDEN AND RELATIVE SIGNIFICANCE OF PRIMARY AND SECONDARY MATERNAL INFECTIONS. A prospective virus isolation study. (II)

Materials and methods. The study comprised 8,367 infants, i.e. four fifths of all newborn infants in Malmö in the 4-year period 1977 - 1981. The infants were studied by virus isolation for CMV excretion in urine within the first week of life (congenital infection). Thirty-five infected infants detected were followed up clinically and virologically together with 51 control infants, who were studied with special regard to postnatally acquired CMV infection. The health status of the congenitally infected infants as determined at birth and during follow-up was related to the type and time of the maternal infection as determined serologically with the CF and IIF-IgM tests.

Results. Of the 8,367 infants studied 38 (0.5 %) showed CMV excretion in the perinatal period. Three isolates from pooled urine samples could not be reisolated from individual samples. Hence, 3 congenitally infected infants could not be identified. Of the remaining 35 infants, 7 (20 %) had symptoms, usually mild, from the reticuloendothelial organs at birth. Two (29 %) of the 7 symptomatic infants and 2 (8 %) of 26 asymptomatic ones with assessable follow-up results at an age ranging from 6 months to 4 years (median 18 months) developed neurologic sequelae attributable to the CMV infection. Developmental disturbances were suspected in all 4 cases already at the age of 3 months, an impaired traction response being then observed. In all 4 cases, severe impairment of hearing was detected at 6 months of age. One of the 4

infants developed a spastic tetraplegia within the first year of life; she was also shown to be mentally retarded. Mental and motor retardation was also detected in another child but the cause might have been constitutional, not virological. In all the cases tested, the radiologic examination of skull and the ophthalmologic examination were normal.

Fifty-one control infants were followed up. By the age of 6 months, 16 (31 %) had acquired a postnatal CMV infection. Four acquired a later infection. Three of the infected infants had recognizable signs of the CMV infection (hepato- or splenomegaly). The control infants developed normally with no signs of hearing impairment in those tested.

Final examinations of the congenitally infected infants and the control infants are scheduled at 7 years of age.

Twelve mothers of the 35 congenitally infected infants studied at birth had antibody patterns characteristic of primary CMV infection and 7 of secondary infection. The remaining 16 women had less conclusive patterns. Most of them had probably a secondary infection during pregnancy (see discussion p.25). The onset of the infections could be specified in 10 cases of primary infection, 3 cases of secondary infection and in further 1 case. Of the primary infections, the 2 cases occurring in trimester I and the single case in trimester II might be specially mentioned.

The 7 infants with CMV symptoms at birth were born to mothers with infections of primary (4 cases), secondary (1 case) or unspecified (secondary?) (2 cases) type. The onset of infection could be established only for the primary type: trimester I (2 cases), II-III (1 case) and III (1 case).

Of the 4 infants with CMV sequelae, 1 (the child with tetraplegia, deafness and mental retardation) was infected due to a primary maternal infection in trimester I. The 3 infants with isolated hearing impairment were infected after a) verified secondary infection at unknown point of time, b) probably secondary infection (see classification p. 15) in trimester II and c) unspecified (secondary?) infection at unknown point of time.

CONGENITAL CYTOMEGALOVIRUS INFECTION. ON THE RELATION BETWEEN TYPE AND TIME OF MATERNAL INFECTION AND INFANT'S SYMPTOMS. (III)

Materials and methods. The study comprised 22 congenitally infected infants and 4 fetuses with a CMV-background, most of them from routine work, as well as 23 congenitally infected infants from the prospective study (II). The aim was to make a comprehensive study of all cases of maternal/congenital CMV infection known by the authors at that time. The cases from routine work increased our possibility to study severe cases of congenital infection. The infants were studied with virus isolation within the first 1 - 2 weeks of life, the fetuses with virus isolation on the organs and the mothers mainly with the CF, ELISA-IgG and IIF-IgM tests but in a few cases also with the ELISA and ELA tests for CMV-IgM activity.

Results. At birth, the central nervous system (CNS) was involved in 5 infants. All 5 also had general symptoms attributable to CMV. General symptoms, such as hepatosplenomegaly, icterus and/or thrombocytopenic purpura, but no signs from CNS, were found in 10 infants. No symptoms attributable to congenital infection were observed in 30 infants.

At follow-up, severe CNS sequelae, such as motoric disability and mental retardation, but also deafness and impairment of vision, were evident in 5 patients. Four of them had neurologic or general symptoms of CMV infection at birth. In the 5th case the causality between the neurological sequelae and the congenital infection was uncertain. Isolated neurologic impairment of hearing was detected in another 5 patients, 3 of whom had general symptoms at birth. Thirty-one children were normally developed at an age ranging from 6 months to 7 years (median 18 months). Two of them were born with signs of CNS involvement (including paraventricular calcifications) and 4 others with general symptoms. Four infants were not followed up: 2 of them died from CID or heart malformation and 2 moved out of town.

Three of the 45 mothers of the live-born infants had characteristic signs of CMV infection (mononucleosis-like disease). Twenty mothers had a serological pattern compatible with primary infection and 5 with a secondary infection. In 20 cases there was an unspecified type of infection. In 5 of the latter cases, the antibody pattern suggested secondary infection (see "classification" page 15).

In the 2 fatal cases of congenital infection, the mothers might have had secondary infections in trimester II or III. In the case of heart malformation, the maternal infection should, accordingly, have occurred after organogenesis. The 5 children with severe CNS sequelae were born to mothers with serologic signs compatible with primary infection in trimester I or II (4 cases) or with unspecified type of infection (the case in which the causality between the CMV infection and the sequelae was uncertain). Isolated hearing impairment occurred after primary infection in trimester I (2 cases), verified secondary (1 case), probably secondary (1 case) or unclassified (secondary? see discussion) (1 case) infections. Congenital infection without apparent sequelae was observed after primary maternal infection in trimester II (2 cases), II-III (7 cases), III (3 cases) and at unspecified time (1 case) and after secondary (4 cases), probably secondary (2 cases) and unidentifiable (secondary? see discussion) infections (12 cases).

Three women with serologic patterns compatible with primary CMV infection in trimester I underwent legal abortion. Two of them had an uncharacteristic respiratory or intestinal disease. No CMV was recovered from amniotic fluid or organs from their fetuses. The third woman had a common cold in gestational month III followed 2 weeks later by thrombocytopenic purpura and haemorrhages. CMV was recovered from several fetal organs but not from the brain. In a 4th case legal abortion was performed for social reasons. The fetus, 19 weeks old, was icteric and had hepatosplenomegaly and ascites. CMV was isolated from the lungs (the only material obtained). Histopathologic examination revealed CMV inclusions in the placenta and liver and inflammatory loci in the kidneys and brain. The type and time of the maternal infection are unknown.

#### RISK OF CYTOMEGALOVIRUS INFECTION IN NURSES AND CONGENITAL INFECTION IN THEIR OFFSPRING. (IV)

Materials and methods. The investigation comprised 292 women working in paediatric clinics or day nurseries and a control group of 163 women who had no professional contact with infants. Sera from student or graduate nurses were obtained within 6 weeks of commencing infant care. In seronegative student nurses, a new sample was obtained 3 months later (more than 6 weeks after completion of the paediatric course). Seronegative graduate nurses were retested after 3, 6 and 9 months, and thereafter at yearly intervals. The seronegative controls were tested once a year. The investigation was per-

formed with the CF and, in some cases, with the IIF-IgM tests.

In a separate study the occupation of 36 mothers of infants with congenital CMV infection was compared to that of control mothers.

Results. Among the women younger than 25, those who had tended infants for more than 6 months were significantly ( $p < 0.001$ ) more often seropositive for CMV than were those - mainly student nurses - with less than 6 months' infant service, but not more often than control women. At ages above 25 there was no intergroup difference in regard to CMV tests. Seroconversion was rare; there was no demonstrable difference between the groups.

Compared to the control group no overrepresentation of nurses was found in the mothers of congenitally infected infants. All 6 congenitally infected infants born to nurses developed normally.

IMMUNITY TO CYTOMEGALOVIRUS IN A NORMAL SWEDISH POPULATION. Comparison with data for pregnant women. (V)

Materials and methods. The material comprised 1,251 sera from patients (626 males, 625 females) attending the Orthopedic Clinic. These patients were considered to represent a normal urban Swedish CMV material. The material had a uniform age distribution from 1 - 78 years; a few individuals were up to 87 years old. For women in fertile age, sera from 171 female blood donors aged 18 - 39 years were added to the material. Results from serological studies of 4,382 pregnant women have previously been reported (I). In all three groups each individual was represented by only one sample. The sera were obtained during the 10-year period 1973 - 1982 and were kept frozen at  $-20^{\circ}\text{C}$ . Serological investigation was performed with the ELISA-IgG.

Results. Forty % (27/67) infants in the age group 1 - 4 years were seropositive. The frequency of seropositives remained fairly constant until the age-groups of 15 - 19 years in females and 20 - 24 years in males. From these age-groups on, there was a successive increase. At ages  $\geq 40$  years, some 85 - 95 % (544/618) of the individuals tested were seropositive.

There was a slight overrepresentation of seropositive pregnant women compared with "normal" women in the age-group 15 - 19 years ( $p < 0.05$ ) but not above this age.



## DISCUSSION

Immunity to CMV in a normal Swedish population. Comparison with data for pregnant women.

About 40 % (27 of 67) of the infants in the normal Swedish population studied were shown to be seropositive to CMV at ages of 1 - 4 years (V). This finding was in good agreement with results from the virus isolation study performed on control infants (II). Few new CMV infections seemed to occur between the age of 5 years and adolescence. After the age of 40, some 85 - 95 % of both males and females were shown to be seropositive (V). The immunity development found was similar to that seen in the USA (5). In developing countries immunity to CMV is acquired at earlier age (55).

The material in Paper V was not suitable for observing the possible disappearance of antibody activity, which has been reported to occur in some cases of CMV infection in infants (2,60) and adults (67). The latter studies were, however, carried out with a less potent antigen. The question of antibody disappearance is of considerable theoretical interest, since later seroconversion could be falsely interpreted as a sign of primary CMV infection.

Unlike the normal population studied, the pregnant women showed a constant frequency of about 72 % (2,898 of 4,046) of CMV-seropositives from 15 to 34 years; after the age of 35 years, 79 % were seropositive (I). The difference between the normal population and the pregnant women was, however, only significant ( $p < 0.05$ ) in the age group 15 - 19 years (V).

Possible explanations of the higher percentage of seropositive pregnant women observed in the age group 15 - 19 years might be an overrepresentation of females at the upper age limit, females of lower social and educational status (63) and/or the presence in this age-group of a larger proportion of immigrants from developing countries known to be seropositive from early age (55). Experience has shown that these immigrants become mothers earlier than Swedish women, whose maximum birth-frequency occurs in the age-group 25 - 29 years.

Frequencies of primary and secondary maternal infections.

Seroconversion was detected in 1.2 % (14 of 1,175) of the seronegative women, who were usually studied from trimester I or early trimester II (I). This finding should be compared to the frequencies of 3 % (8 of 254) seroconverters in seronegative white women studied from trimester I by Stern et al (66), 0.9 % (14 of 1,608) in trimester II or III found by Griffiths et al (26) and

0.6 % (4 of 664) within the late 2nd and 3rd trimesters found by Monif et al (47). Stagno et al (63) diagnosed 1.4 % (17 of 1,203) cases in an upper income group and 2.2 % (4 of 179) in a low income group enrolled before the 18th week of pregnancy. Seroconversion is generally considered to indicate primary infection, although objections might be raised against this statement as mentioned above.

Pregnancy has been considered to enhance the reactivation of latent CMV infection: frequencies of 0 - 1.6 % have been demonstrated in the 1st trimester and 8 - 15 % in the 3rd trimester (5). However, Stagno et al (59) found that early pregnancy appears rather to exert a suppressive effect on viral excretion which wanes with advancing gestation. Pregnant and non-pregnant women with similar demographic characteristics were shown to have identical overall rates of cervical CMV excretion (9.5 % vs. 9.4 %).

We did not study the frequency of secondary (reactivated latent or reinfection) infections in mothers. Such an investigation must be made with the cumbersome virus isolation technique, since these infections are generally not detected serologically (25). The last observation is supported by our findings that only 3 of 7 women with antibodies before pregnancy and 1 of 16 with the pattern of positive IgG and negative IgM tests at the first antenatal control had demonstrable IgG or IgM titer rise with the CF, ELISA-IgG or IIF-IgM tests. The women were mothers of congenitally infected infants (II).

We found, however, indirect evidence of frequent occurrence of maternal CMV infections around delivery: 31 % (16 of 51) control infants, all born to seropositive mothers, acquired postnatal CMV infections within the first 6 months. It is generally accepted that peri- and neonatally acquired CMV infections are mainly acquired from the mothers: the CMV can be transmitted with infected cervix or throat secretions or milk.

#### Transmission of primary and secondary maternal infections.

Primary maternal infection was shown to be transmitted to the fetus in 43 % (6 of 14) cases, a frequency similar to those found by Stern et al (45 % or 5 of 11) (66), Nankervis et al (50 % or 4 of 8) (48) and Stagno et al (52 % or 11 of 21) (63). Griffiths et al found transmission in 25 % (3 of 12) (26).

Transmission is possible during the entire pregnancy period as shown by us and others (II,III,66). Placental CMV infection, without fetal involvement, has been described by Hayes et al (32). Griffiths et al (27) found a significant excess of fetal death in women primarily infected with CMV; there was no histological evidence that the fetuses had become infected; they

suggested that the fetal death might have resulted from placental insufficiency.

From data in the literature, Medearis (45) estimated that only 6 % of infants at risk for acquiring a congenital infection from a secondary maternal one actually do so.

### Congenital infection

CMV is the most common cause of congenital viral infection, with an average incidence of 1 % of all live births (63). In our study (II), we found a frequency of 0.5 % (38 of 8,367) which should be compared to the 0.4 % (12 of 3,060) found in Denmark (7). Low socioeconomic standard has been shown to be associated with a higher frequency of congenital infections (63).

We found that 20 % (7 of 35) congenitally infected infants had symptoms at birth from the reticuloendothelial organs, such as hepatomegaly, splenomegaly and/or thrombocytopenic purpura (II). The symptoms were, however, sometimes so mild that they were not even mentioned at discharge from the Obstetric Clinic. The frequency of neonatal symptoms might therefore be considered comparable to the 9 % (6 of 68) found by prospective virus isolation (79,41,46,64,65,66) (Table 2, Paper II). Also Stagno et al (63) consider that few (5 to 10 %) of the infants infected have symptoms ranging from fatal to moderate at birth.

It is generally agreed that symptoms at birth, whether of neurological or reticuloendothelial type, are bad prognostic signs. Death is rare, but high frequencies (>85 %) of neurological sequelae occur after these initial symptoms (30). Stagno et al in Alabama (63) are also of the opinion that late complications almost invariably occur in survivors of the 5 - 10 % congenitally infected infants with neonatally symptomatic infection. This tendency was confirmed in one of the present studies (III) in which 60 % (3 of 5) of the infants who had CNS signs at birth and 50 % (4 of 8) of those who had symptoms from the reticuloendothelial organs had neurologic sequelae after an observation period ranging from 6 months to 8 years (median 2 years). In contrast, only 8 % (2 of 26) infants without symptoms at birth developed neurologic sequelae after a follow-up period ranging from 6 months to 4 years (median 18 months) (II). In an early review from Alabama (4) neurologic complications were considered to be relatively common also in neonatally asymptomatic infants, but in a later paper (63) the authors suggested the risk to be 10 %.

Our total finding of 12 % (4 of 33) infants with sequelae in the prospective study (II) should thus be similar to the discoveries by the Alabama group (63). The risk for minor sequelae and sequelae that have not yet appeared will be judged when the infants' development is finally reviewed at 7 years of age.

The 4 cases of neurological sequelae found in the prospective study (II) would be equivalent to an estimated frequency in the general Swedish population of 0.06 %. With 100,000 newborns a year, some 60 Swedish infants yearly should develop more or less severe neurologic sequelae, mainly deafness, from congenital CMV infection.

The 4 infants with deafness constituted the major part of the otoneurologic injuries detected in infants within the region and period studied.

#### Type of maternal infection in case of congenital infection in the infant.

When the prospective study was planned, there was no evidence in the literature that secondary maternal CMV infection should be more than an exceptional cause of congenital infection: a few cases of congenital infection in siblings from consecutive pregnancies had been reported (18,40). But soon after the start of the study, two papers presented evidence of secondary maternal infection as a common cause of congenital CMV infection (55,61).

The present serological analyses of the mothers of the congenitally infected infants classified them into 3 groups: those with primary infections (seroconversion or high level of CMV IgM, i.e. IIF-IgM titer  $\geq 64$ , in the first postconceptional serum), those with secondary infection (CMV antibody activity before pregnancy) and those with unclassifiable infections (CMV-IgG but no CMV-IgM in the first postconceptional serum and no serum sample from the preconceptional period). The latter women could be divided into 2 subgroups: those with subsequent IgG or IgM titer rise (probable secondary infection) and those without.

Since CMV-IgM antibodies were shown to persist for 1 - 6 months in 10 control patients with CMV infection (III) and for 3 - 6 months in 2 of 3 primarily infected pregnant women followed-up (II) and (with one exception) to be an invariable phenomenon in mothers with seroconversion (I), the absence of specific IgM in trimester I or early trimester II was considered to exclude primary infection in early pregnancy with high probability. Therefore, most of the women with unclassifiable infection might have had a secondary infection. Accordingly, up to two thirds of the congenitally infected infants

studied prospectively might have been infected due to secondary infections in the mothers.

In developing countries, practically all congenital CMV infections can be attributed to secondary maternal infections since most women in fertile age are seropositive to CMV (55). In the USA, secondary infections were shown to be more important in a low-income class than in a high-income one (63).

Relation between the type and time of maternal infection and the outcome of the offspring.

The relation between maternal infection and congenital CMV infection/disease was investigated in two materials, one prospectively studied and dominated by neonatally asymptomatic infections in the infants (II) and the other, partly selected in routine work, containing a higher proportion of infants with neonatal symptoms (III). According to the first of these studies, 2 of 3 primary maternal infections in trimester I or II caused neonatally symptomatic infections, but only 1 (an infection in 1st trimester) gave permanent neurologic sequelae in the infant (tetraplegia, mental retardation, deafness). A further 3 infants with permanent neurologic sequelae (deafness) were detected. Their symptoms were attributable to verified secondary, probably secondary or unclassifiable (secondary?) maternal infections. Only the "probably secondary" infection could be dated: it occurred in 2nd trimester. Conversely, 1 of 12 mothers with primary infection gave birth to a congenitally infected infant with neurological sequelae and 3 of 23 mothers with more or less certain secondary infections had congenitally infected infants with neurological sequelae. The proportions (1 of 12 and 3 of 23) were comparable, but primary infection caused more severe injury (tetraplegia, deafness, mental retardation as compared to isolated deafness). The considerable risk of severe infant disease following primary maternal infection in trimester I (and II) was illustrated in Paper III. However, it can not be excluded that also secondary maternal infection might cause severe disease in the infant as shown by a fatal case of CID (III).

In their study (63), the Alabama group of authors found that 5 of 33 congenitally infected infants born after primary maternal infection had apparent disease in the neonatal period. One of the 5 died and 1 survived with profound psychomotor retardation. Of 27 congenitally infected infants born to mothers with secondary infection, none had symptoms at birth. The health status at birth was significantly different in the 2 infant groups. The follow-up was too short to determine whether there were also differences in



the risk of delayed sequelae between infants whose mothers had primary infection and those whose mothers had secondary infection.

Because of the small number of infants with sequelae and the short follow-up in both materials it is impossible to compare the findings in the Malmö (II) and the Alabama (63) studies. It must, however, be emphasized that the Malmö material has shown that secondary maternal infection can cause neurologic sequelae (deafness) in the infant. This is a principally important fact, which has implication on vaccination. Some earlier data have been suggestive in this respect: Yeager et al (72) reported on 2 siblings born 1 year apart and both congenitally infected with CMV. The 2nd child developed neurologic disease - seizures - at 7 months of age. Hayes et al (33) described an immunodepressed woman with preconceptional antibodies to CMV, who gave birth to a neonatally asymptomatic infant excreting CMV. Later the infant developed severe brain damage. But prolonged labour and delivery by forceps may also have contributed to the brain damage.

#### Prevention of congenital infection/disease

The possibility of maternal re-infection and the common transmission of CMV to the fetus after secondary maternal infection indicate that vaccination of fertile women would have a questionable effect on the frequency of congenital CMV infection. It is a matter of course that a live vaccine strain must not be able to give latent infection with risk for the fetus. Possibly, vaccination would modify the clinical appearance of the congenital infection. More knowledge of the influence of secondary maternal infection on the infant's long-term development is necessary.

Would routine serological screening of pregnant women for the purpose of interrupting "risk pregnancies" be a reliable alternative to vaccination? The diagnosis of primary maternal infection, on which most interest would be focused, would for natural reasons often be founded on the finding of CMV-IgM in the first antenatal serum sample. A positive IgM test would, however, give less exact information on the type and time of maternal infection than a seroconversion found in paired acute and convalescent sera. Such paired sera would as a rule only be obtainable after the first antenatal visit. In any case, a confirmatory test of accomplished fetal infection would be desirable such as virus isolation on amniotic fluid. But this test has only been tried with positive results in a few cases (15,21) and the experiences of the method is still limited.

It is unavoidable that serological screening in many cases would give diffi-

cult decisions and worry for the women tested. The cost for the screening would be high. The following must also be considered: some primary infections result in spontaneous abortion (27); only 50 % of them are transmitted to the fetuses (I,48,63,66); only some 10 - 20 % of those infected will get neurological sequelae (II,63), most of them of limited extent (II); screening would probably detect the most severe cases but miss up to 50 - 75 % of the total number of infants with sequelae (II,III). Time seems not mature for routine serological screening. More experience of the diagnostic and clinical problems of maternal and congenital CMV infections is necessary.

## CONCLUSIONS

1. The frequency of congenital CMV infections in a Swedish material was shown to be 0.5 %. Of the congenitally infected infants, 20 % had (usually mild) symptoms at birth; 29 % of the symptomatic infants and 8 % of the asymptomatic ones or totally 12 % had neurological sequelae at an age ranging from 6 months to 4 years (median 18 months) (II). This would be equivalent to a frequency of 0.06 % in the general Swedish population or some 60 Swedish infants a year, the majority of them with isolated hearing impairment. Further follow-up is necessary before final judgement.
2. Of the pregnant women studied, 72 % were shown to be seropositive to CMV. Of the seronegatives, 1.2 % acquired a primary CMV infection after the first antenatal visit which usually was performed late in trimester I or early in trimester II. In 43 % the primary infections were transmitted to the fetuses (I).
3. One third of the congenital infections diagnosed could be attributed to primary maternal infections. It was shown that also secondary maternal infection can be transmitted to the fetuses: up to two thirds of all the congenital infections could be attributed to secondary infections in the mothers. Infant disease was found to occur after both primary and secondary maternal infection (II). Primary maternal infection in first (or second) trimester seemed to be the qualitatively most serious threat to the infant (II,III), but quantitatively the secondary maternal infections seemed to play an equally important role (II).
4. Prospective CMV-IgM and -IgG analyses of maternal sera during early pregnancy would have predicted congenital CMV infection with neurological sequelae or fatal outcome in 25 - 50 % of the cases diagnosed (II,III).
5. We found little or no evidence that nurses should be at greater risk for acquiring CMV infection than other women.

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## PRIMARY AND SECONDARY MATERNAL CYTOMEGALOVIRUS INFECTIONS AND THEIR RELATION TO CONGENITAL INFECTION

### *Analysis of Maternal Sera*

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**ABSTRACT.** Ahlfors, K., Ivarsson, S.-A., Johnson, T. and Svanberg, L. (Departments of Clinical Virology, Paediatrics and Obstetrics and Gynecology, University of Lund, Malmö General Hospital, Malmö, Sweden). Primary and secondary maternal cytomegalovirus infections and their relation to congenital infection. Analysis of maternal sera. *Acta Paediatr Scand*, 71: 109, 1982.—4382 new mothers were examined retrospectively with the enzyme-linked immunosorbent assay (ELISA) for IgG activity to cytomegalovirus (CMV) during pregnancy. Some of them were also studied with the indirect immunofluorescence (IIF) test for CMV-IgM antibodies. All the infants had been studied for CMV excretion within the first week of life. Nineteen of them had been shown to be congenitally infected with CMV. 1218 (28 %) women lacked CMV-IgG activity at their first antenatal visit (usually in months III-IV). Fourteen of them seroconverted before parturition (primary infection). Thirteen of the seroconverters were shown to develop CMV-IgM activity. In 6 (43 %) cases the primary infection was transmitted to the offspring. The remaining 13 congenitally infected infants were born to mothers with a positive IgG-test at their first antenatal control. Only one of these mothers had a clearly positive IgM-test. She was shown to lack CMV-antibodies before conception (primary infection during the first trimester). Preconceptional sera were obtained from further 4 of the 13 seropositive mothers of congenitally infected infants; all 4 had CMV antibodies before pregnancy (secondary infection during pregnancy). The combined studies of the mothers and infants revealed that 21-63 % of the congenital infections could have been caused by secondary maternal infections. Prospectively performed, the study would only have disclosed one of the three fetal CMV infections that resulted in neurological sequelae.

**KEY WORDS:** Cytomegalovirus, maternal infection, congenital infection, serology, enzyme-linked immunosorbent assay (ELISA)

The present study is a part of a prospective investigation of problems bearing on congenital cytomegalovirus (CMV) infection (1-4). It concerns a retrospective serological survey of mothers whose infants were studied for CMV excretion in the perinatal period (1, 2). The size and the composition of the infant and maternal materials were considered such as to warrant estimation of the proportion between congenital CMV infections caused by primary and secondary (reactivated latent or re-infection) maternal infections.

## MATERIALS AND METHODS

### *Clinical material*

**Mothers.** The material consisted of 4382 mothers of infants studied for CMV excretion in the urine within the first week of life. The study covered four-fifths of all new mothers within a 2-year period, 1977-79, in the city of Malmö with some 225 000 inhabitants. Because of missing or unsatisfactory virus isolation tests of urine from their infants, one-fifth of the women were not investigated. Thus, among others, mothers of stillborn offspring and of infants who had died before urine had been sampled, were

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† We regret to record the death of Professor Johnson during the publication of this article.



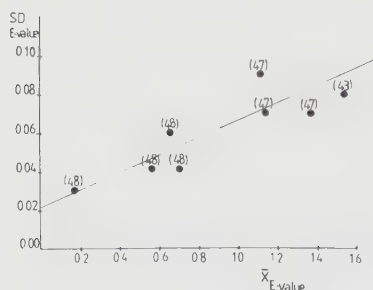


Fig. 1. Within assay precision determined by repeated tests of eight sera, each on the same polystyrene plate.  $\bar{X}_{E\text{-value}}$ : mean  $E$ -value at 405 nm,  $S.D._{E\text{-value}}$ : standard deviation. Figures in brackets denote number of tests. The straight line was obtained by linear regression analysis.  $r$  (correlation coefficient) 0.89 ( $p=0.001$ ),  $y$  intercept 0.02, slope 0.045.

excluded. Combined clinical and pathological-anatomical data on the dead infants gave no evidence of cytomegalic inclusion disease (CID) in any of them.

**Maternal sera.** For several years blood samples have regularly been obtained from pregnant women in connection with their first antenatal control as well as at delivery. Since the beginning of the present study also a number of intermediate sera were sampled. In addition, samples obtained in connection with infectious diseases other than CMV were sometimes available.

**Infants.** Among the virologically tested 4421 infants of the above-mentioned mothers, 19 (0.4%) had a positive CMV isolation test within the first week of life (2). The smaller number of mothers than infants investigated was due to twin births and missing sera.

#### Laboratory materials and techniques

**Antigens.** Both the enzyme-linked immunosorbent assay (ELISA) and the complement fixation (CF) test antigens were prepared according to a procedure described by Krech & Jung (5), as modified by Cremer et al. (6), namely extraction of CMV-AD-169-infected human embryonal lung fibroblasts with alkaline glycine buffer. Control antigen was prepared in the same way from uninfected cells. For the indirect immunofluorescence (IIF) test for IgM-antibodies the antigen was prepared according to a previously described technique: CMV-AD-169-infected human embryonal lung fibroblasts were grown on slides and fixed in acetone (7). The ELISA and the CF antigens were stored at  $-70^{\circ}\text{C}$  and the IIF antigen at  $-20^{\circ}\text{C}$  until use.

**Sera.** All the sera were stored at  $-20^{\circ}\text{C}$ .

**Conjugates.** An alkaline phosphatase conjugated swine anti-human immunoglobulin G ( $\gamma$ -chain specific) (Orion Diagnostica, Finland) was used for the ELISA and a FITC-conjugated rabbit anti-human immunoglobulin M ( $\mu$ -chain specific) (Dakopatts A/S, Denmark) for the IIF.

**ELISA for IgG antibodies.** The micromethod of Voller et al. (8) as used by Vejtorp (9) in the diagnosis of rubella was followed with the exception that the incubation temperature ( $35^{\circ}\text{C}$ ) was thermostatically controlled. The colour intensity was measured at 405 nm by an automatic spectrophotometer (Titertec, Multiskan, Flow Laboratories, Irvine, Scotland). The absorbance of samples, expressed as  $E_{405}$ , was automatically recorded relative to that of control samples containing substrate, buffer and NaOH. The antibody content ( $E$ -value) was expressed as the  $E_{405}$  difference in tests performed with serum against virus and control antigens. A titrated positive control serum and a negative control serum diluted 1/40 were included in the runs. Virus antigen dilution 1/50 was preferred because it gave a practically optimal  $E_{405}$  for the positive reference serum and a low non-specific binding for the controls. Conjugate dilution 1/50 had an almost optimal effect. Patients' sera were tested in dilution 1/40.

Eight sera with mean  $E$ -values between 0.17 and 1.54 were repeatedly tested on one day (Fig. 1). The positive reference serum was tested in serial dilutions on 10 consecutive occasions. In the linear part of the S-shaped curve ( $E$ -values of 0.2–1.0) the interday variations were at most of half the size of a 10-logarithm. Of 1000 consecutive maternal sera studied, the  $E$ -value distribution was:  $<0.10$  (27.7%), 0.11–0.20 (3.4%), 0.21–0.50 (1.2%), 0.51–1.00 (6.2%) and  $>1.00$  (61.4%). The mean  $E_{405}$  against negative antigen was 0.04. We decided to consider  $E$ -values of  $>0.20$  as positive.

**CF test.** The micromethod described by Sever (10) was used. A titre of  $\geq 8$  was regarded as a positive reaction and a fourfold or larger titre increase as significant.

**Comparison of ELISA and CF test.** 685 sera first tested with ELISA for CMV-IgG were reinvestigated with CF. Of 533 sera with  $E$ -values of  $\leq 0.20$ , 531 had a CMV-CF titre of  $<8$ ; the remaining two sera had a CF titre of 8. Of sera with  $E$ -values of 0.21–0.50, 0.51–1.00 and  $>1.00$  36/40, 58/60 and 52/52, respectively, had a CF titre of  $\geq 8$ . Increasing  $E$ -values were regularly accompanied by increasing CF titres. However, interindividually, the relation between the  $E$ -value and the CF titre varied considerably.

**Cross-reactions with other herpes viruses.** A few patients with active herpes simplex, varicella or Epstein-Barr virus infections were tested with the CMV-ELISA-IgG. No cross-reactions were observed.

**IIF test for IgM antibodies.** As reported elsewhere IIF-IgM titre 64 was considered a positive borderline value indicating an active CMV-infection within the last one to six months (3). A four-fold or greater titre change was also considered to indicate active infection. IgM-positive sera were tested for rheumatoid factor (RF) with Latex-RF-Reagenz (Behringwerke, West Germany) (11). If the RF test was positive the sera were retested after absorption with aggregated human gammaglobulin (12).

## RESULTS

### ELISA performed on maternal sera

Of the 4382 maternal sera obtained in connection with the first antenatal visit, 1218 (28%)

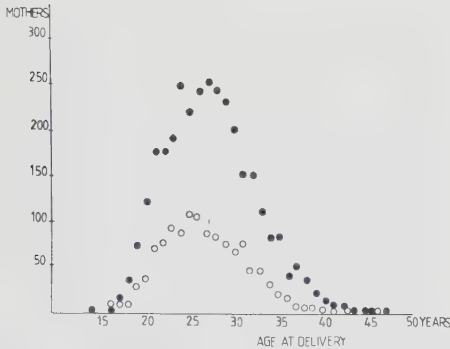


Fig. 2. Age distribution of the seropositive (●) and the seronegative (○) mothers at delivery.

lacked IgG activity to CMV antigen. The percentage of seropositive women in each 5-year group between 15 and 34 years of age was 71–72. After the age of 35 years 79% were seropositive. The age distribution is shown in Fig. 2. Samples obtained at delivery of 1175 previously seronegative women showed an ELISA-IgG seroconversion, verified by CF test, in 14 (1.2%) cases. Conversion was considered a sign of primary infection. The seronegative women were followed up (usually from months III or IV until delivery) for altogether 595 years.

#### *Seronegative women*

##### *Congenitally infected infants*

The 14 women with CMV-seroconversion between the first antenatal visit and delivery were 20–35 (median 27) years old at parturition. Seven of them were primiparous. None was suspected of having CMV disease during pregnancy. Six (43%) of the 14 mothers gave birth to infants congenitally infected with CMV, as shown by positive virus isolation test within the first week of life. In the remaining 8 cases the virus isolation test was negative. (The development of these 8 infants was normal at the age of  $\geq$  one year.)

By studying intermediate sera the seroconversion of the 6 mothers with infected infants

could be dated more closely to months III–X, IV–VI, V–IX, VI–IX, VI–IX and VII–VIII, respectively. The corresponding times for the mothers with uninfected infants were months III–IX, III–X, IV–IX, VI–VIII, VI–IX, IX–X, IX–X and IX–X. Thirteen of the 14 mothers had also experienced a significant titre rise in the IIF-IgM test. In 6 of the cases the IgM titre was  $\geq 128$  (highest titre 1024) and in another 6 cases it was 64. In the 13th case there was only an increase from titre  $< 10$  to 40. In the 14th IgM-negative case the two samples showing IgG seroconversion had been obtained 3 months apart.

#### *Seropositive women*

##### *Congenitally infected infants*

Thirteen of the infected infants were born to mothers with CMV-IgG activity (ELISA) in their first antenatal serum sample. The mothers' ages varied between 21 and 34 (median 26) years; 3 were primiparous. The CMV infection was invariably subclinical. Twelve of the 13 mothers had a stable, positive CMV-IgG titre between the first antenatal check-up (usually in months II–IV) and parturition. The 13th woman had a significant increase in her positive IgG titre between months IV and VI without significant CMV-IgM activity. One of the mothers with a constant IgG had a high IgM titre of 320 in month III. A second woman had a permanently low IgM titre of 40 from month IV until delivery. In 5 cases preconceptional sera were available. Four of them contained CMV-IgG antibodies; but not the fifth, which was from the mother with an IgM titre of 320 in month III.

## DISCUSSION

In the present study the CMV-ELISA, previously tried in small series (13–18) proved a simple and reliable method for demonstrating IgG antibodies. The agreement with CF was extremely good, although it is known that the CF test can also detect CMV-IgM antibodies (19). One advantage of ELISA was that anti-

complementary factors did not disturb the test and that determination of the antibody content with good precision required only one serum dilution. ELISA would apparently be useful in large-scale epidemiological surveys for demonstrating CMV-IgG activity.

The 14 seroconversions after the first antenatal visit corresponded to a frequency of 0.3% primary infections during trimesters II and III. Stern & Tucker (20) found seroconversions in 1.4% between the first trimester and delivery, while Monif *et al.* (21) found only 0.06% within the late second and the third trimesters. The almost 50% transmission frequency of primary maternal CMV infections to the offspring in the present material agreed with the figures presented by Stern & Tucker and by Nankervis *et al.* (22); but Monif *et al.* found a 100% transmission frequency. Our material did not permit any conclusions about the transmission during different trimesters. According to Stern & Tucker, transmission is more common during early pregnancy, but Nankervis *et al.* found the highest frequency in trimester III.

Out of the 19 congenitally infected infants 7 (37%) were born to mothers with primary infection, which had occurred in one of them before, and in 6 after the first antenatal visit. Twelve congenitally infected infants were born to mothers without signs of primary infection. In 4 of the latter CMV-IgG was shown in samples taken before conception. Accordingly, at least 4 (21%) infants must have been infected due to a secondary maternal infection.

For obvious reasons (missing preconceptional sera) secondary maternal infections are usually difficult to diagnose. Possibly, more such infections might be concealed among the 12 mothers of infected infants with CMV-IgG antibodies at the first antenatal control and who lacked significant IgM activity. Thus, some 4–12 (21–63%) of the intra-uterinely infected infants could have contracted CMV from a secondary maternal infection.

Maternal CMV infection is usually subclini-

cal. Screening women for primary infection in the first or second trimester would require that all mothers be tested for IgG antibodies at their first antenatal visit and that the IgG-positive women be tested further for specific IgM antibodies and the IgG-negative ones be retested for seroconversion before week 20, i.e. the time limit for legal induction of abortion in Sweden. A prospective serologic study performed on the present material would have revealed only 2 cases of congenital infection due to primary maternal infection before week 20. In the first case the mother had a primary infection before month III (seroconversion at comparison of a preconceptional serum sample and a sample from month III and a CMV-IgM titre of 320 in the latter sample). The infant, who is now 1½ years old, is deaf and has spastic tetraplegia. In the second case the mother had a primary infection in months IV–VI (seroconversion and positive IgM test). The infant is now one year old and normally developed.

Possibly, 2 further cases of primary infection resulting in congenital infection would have been revealed if more sera had been obtained from all seronegative women close to pregnancy week 20. The maternal infection in these 2 cases was dated to months III–X and V–IX, respectively. Both infants, now more than one year old, are normally developed.

Regular screening of all pregnant women would also have revealed primary infections not resulting in congenital infection as well as low IgM titres of inconclusive value (23).

Besides the above-mentioned child, born to a mother with primary infection in trimester I, 2 other congenitally infected infants have developed neurological sequelae. One of them was born to a woman in whom an already positive CMV-IgG titre increased further in months IV–VI. Since no significant IgM activity was detected in the first serum sample the infection was interpreted as possibly secondary (reactivated latent or re-infection). The infant has severe sensorineural impairment of hearing. The third infant with neurological se-

quelaе (sensorineural hearing impairment of severe degree) was born to a mother with an unknown type of infection (stable IgG and no IgM activity from month III).

Judging from the present investigation, it would hardly be meaningful to screen all pregnant women for primary CMV and still less so for secondary infection. Only one out of 3 infants hitherto found to have neurological sequelae would have been considered at risk.

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FREQUENCIES OF CONGENITAL CYTOMEGALOVIRUS INFECTION AND DISEASE IN SWEDEN  
AND RELATIVE SIGNIFICANCE OF PRIMARY AND SECONDARY MATERNAL INFECTIONS

A prospective virus isolation study

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## ABSTRACT

8,367 newborn infants were investigated by virus isolation for cytomegalovirus (CMV) in the urine at birth. Congenital infections were found in 38 (0.5 %). Of 35 infected infants studied at birth, 7 (20 %) had (usually mild) symptoms referable to the reticuloendothelial organs. Two (29 %) of the 7 symptomatic infants and 2 (8 %) of 26 asymptomatic infants or totally 4 (12 %) of the 33 infants with assesable follow-up results had neurologic sequelae attributable to CMV at the age of 6 months to 4 years (median 18 months). Developmental disturbances were suspected in all 4 cases already at the age of 3 months. The 4 cases of sequelae would be equivalent to an estimated frequency in the general population of 0.06 %. Twelve mothers of the 35 infants studied at birth had serological signs of a primary infection, 7 of a secondary (reactivated latent or re-infection) and 16 of an unspecified (possibly usually secondary) infection. One of the above 4 injured infants who was mentally retarded, tetraplegic and deaf was born to a mother with primary infection in the 1st trimester. The remaining 3 infants with sequelae, all with severe deafness, were born to mothers with proven, probable or possible secondary infection, respectively; all 3 infants were mature for age and had a good communication ability. Prospective studies of maternal sera would have suspected only the above mentally retarded infant to be at risk.

KEY WORDS: Cytomegalovirus, infection, congenital, maternal

## INTRODUCTION

The majority of congenitally infected infants are asymptomatic at birth, but some have symptoms referable to reticuloendothelial organs or, though rarely, to the central nervous system (CNS). All infected infants, irrespective of their status at birth, are believed to be at risk for neurologic sequelae (13). It is usually not possible to establish the relation between the mother's type and time of infection and the infant's infection, symptomatic or not. Both primary (31) and secondary (25,26) maternal CMV infections can cause infant infection. Disease of the infant has been referred mainly to primary maternal infections in trimester I or II (6,21,31), less often to secondary maternal infection (4,6,14). Preventive measures require better knowledge of the frequency of congenital disease and the significance of primary and secondary maternal infections. The only reliable method to get increased experience is a large-scale prospective study of all mothers and infants in non-selected materials.

## MATERIALS AND METHODS

The infants studied were born in the city of Malmö, Sweden, within a 4-year period (1977-1981). The town has 225,000 inhabitants, 2,500 deliveries a year, a single hospital for somatic diseases, and laboratories situated close to the clinics. The majority of the pregnant women in the town attend the antenatal clinic at the hospital and all deliveries take place in the hospital. It is thus possible to trace most pregnant women and all newborns. For several years sera have been obtained from all pregnant women at their first antenatal control and at delivery. From the beginning of this study sera were also obtained one or several times in between. This enabled both pre- and postconceptional serological studies in multiparae. In primiparae the preconceptional serological status could be determined only in case serum had been obtained in connection with a previous infection.

For several reasons (differences in the extent of the studies of the mothers, variations in the virus isolation technique and different follow-up periods) the results are presented in 2 parts, representing one 2-year period each. The description of the material refers to March 1982, when the youngest infected infant was 6 months old.

Infants. All together 8,367 live-born infants, approximately four fifths of all, were adequately tested, that is, urine samples were obtained within the first week of life and maintained in culture for 4 weeks. The remaining fifth were missed mainly because of technical mishaps at the laboratory (batches of inadequate cell cultures). The only infants more or less regularly missed at the clinic were those referred to special departments immediately after birth for surgery of life-threatening malformation or respirator treatment. Of 78 infants who died intrauterinely at the gestational age of  $\geq 28$  weeks or postnatally within the first week of life (in Malmö), urine, lung, kidney and/or brain material from 38 were studied for CMV. All 78 were examined post mortem by pathologists. Congenitally infected infants and control infants, two per index case, were followed up clinically and for CMV excretion. The only conditions to be met by a control infant were that it had not had CMV at birth and that it was born in a specific order relative to the index case. Twenty-three of the patients have been included in previous publications (3,4,5,6).

Mothers. In the first 2 years 4,382 mothers of 4,421 infants tested were examined for antibodies to CMV (5). In this study the results of serological studies on the mothers of infected infants and of control infants are presented. Later only mothers of infected infants were tested.

Virus isolation. During the first 2-year period all the urine samples were tested separately. During 9 months in the second 2-year period pools of 5 samples were used. The inoculated volume was 0.2 ml consisting of urine in transport medium. This corresponded to an approximate volume of 0.1 ml urine (0.02 ml of each sample in the pools). CMV was isolated in cultures of local strains of human embryonal lung fibroblasts (1,3). The virus was indentified by its typical cytopathic effect (CPE) and failure to replicate in epithelial cells (in the whole study period) and by immunofluorescence (in the first 2 years). For virus titration fresh urine in transport medium was serially diluted 1/10 in phosphate buffer. From each dilution 0.2 ml, corresponding to about 0.1 ml urine in first step, was inoculated into each of two tissue culture tubes. Titers are expressed as TCID<sub>50</sub> of virus per 1 ml urine.

Serologic tests. CMV antibody activity was studied with the complement fixation (CF) test (alkaline extracted CMV-AD-169 antigen), and specific IgG

activity with the enzyme-linked immunosorbent assay (ELISA) (same antigen as in CF). The indirect immunofluorescence (IIF) test was used for the demonstration of specific IgM (CMV-AD-169 infected fibroblasts grown on slides). To avoid false positive reactions caused by rheumatoid factors IgM positive sera were retested after absorption with aggregated gammaglobulin. The methods used have been described previously (2,5).

Clinical investigation. All newborns were examined (birth weight, length, head circumference, Apgar score and general somatic status) by paediatricians. Thirty-four of 35 infants infected and 51 control infants were followed up (by SI) for general development and neurologic status at the age of 3, 6, 9 and 12 months and 1 1/2, 2 1/2 and 4 years (15,32). In both groups of infants age at last control ranged from 6 months to 4 years with a median of 18 months. Hearing was examined (by SH) in 30 patients and 46 controls at the age of 1/2, 1, 2, 3 and 4 years with a median age at last control of 2 years for both groups. The following tests were used: APR, observation tests (pure tones, informal sounds, BOEL-test), peep-show-audiometry and play-audiometry. Children with suspected or confirmed hearing loss were examined also with neuro-physiological tests (ECoG, brainstem audiometry, cortical evoked response audiometry) (17). Twenty-eight patients aged 6 months to 2 1/2 years (median 7 months) were examined ophthalmologically with special reference to chorioretinitis. Twenty-nine infants infected aged 5-11 months were examined radiologically (by GT). The examination comprised assessment of intracerebral calcifications and cranial size (index method devised by Cronqvist) (11). At an age ranging from 20-27 months (median 22 months) 23 infected infants were examined psychologically (by RH and BL) according to Griffiths' mental development scale (12), adjusted to Swedish standard for the age of 0-2 years by Lindstam (19).

It is planned to review the children at 7 years of age.

Controls of infants with congenital infection and sequelae. In case of neurologic sequelae in an infant congenitally infected with CMV other congenital infections (rubella, toxoplasmosis, syphilis) were excluded serologically.

## RESULTS

Virologic survey of the infants. In all, 38 (0.5 %) positive isolates were found in the 8,367 samples adequately examined. In the first 2 years the number of positives was 19 (0.4 %) of 4,421 samples; in the next 2 years, 19 (0.5 %) of 3,946 samples. Three positive isolates found in pools of urine samples could not be reisolated from individual samples. These isolates were considered to represent one infected infant each. CMV finding within the first week of life was considered sign of congenital infection. Of the 35 congenitally infected infants identified, 16 were girls and 19 boys.

Congenitally infected infants. The virus excretion was graded according to CPE as abundant (+++), medium (++) or sparse (+). The CPEs corresponded to approximative titers of  $10^{-4}$ - $10^{-6}$ ,  $10^{-2}$ - $10^{-4}$  and  $10^{-1}$ - $10^{-2}$ . The (+++) CPE appeared at the 1st - 2nd, the (++) at the 2nd - 3rd and the (+) at the 2nd - 4th weekly inspection. No new isolate was found at the last inspection. Eighteen (51 %) infants, including all the 4 with verified CMV sequelae (see below), excreted CMV abundantly at birth, 14 (40 %) moderately and 3 (9 %) sparsely. Primary maternal infection (see below) caused massive excretion in 9 (75 %) of 12 infants and secondary maternal infection in 3 (43 %) of 7 infants.

The frequency of virus positives and abundant excretors decreased with increasing age. At 30 months only 6 of 10 children studied excreted the virus. Seven infants had still a massive excretion at 6 months and 1 at 12 months. After that age none of the 11 children studied was found to have an abundant excretion.

Clinical appearance of congenitally infected infants at birth. All the 35 infants but one were born at term; one was small for gestational age. None had neurologic symptoms, 7 had characteristic, usually mild, symptoms referable to reticuloendothelial organs (hepatosplenomegaly and/or thrombocytopenic purpura) and 28 were asymptomatic or had non-characteristic symptoms. One infant was transmitted to the Paediatric Clinic because of its typical CMV symptoms and one because of low weight.

Follow-up of congenitally infected infants. One infant moved from the town soon after birth. None of 34 infants followed up died. Of the 19 infants from the first 2-year period, 3 had obvious sequelae attributable to CMV when last examined at 1 1/2 - 4 years of age. One had mental retardation,

spastic tetraplegia and deafness and the remaining 2, severely impaired hearing. In all 3 cases sequelae were first suspected at the 3-month control: a (reversible) impairment of the traction response with maximal head lag was noticed; severe deafness was diagnosed at the 6-month control; the symptom of tetraplegia was evident within the first year of life. In addition, in one infant (the only one small for gestational age) mental and motor development was possibly retarded, but not due to CMV with certainty. Of the injured infants, only the girl with tetraplegia had symptoms of CMV (petechiae but no apparent neurological signs) at birth.

Of the 16 infants born in the second 2-year period, 15 were followed up for 6-18 months. Only one of them had obvious sequelae. He had hepatosplenomegaly and petechiae at birth, (reversible) impairment of the traction response at 3 months and severe deafness detected at 6 months of age.

None of the children tested ophthalmologically showed signs of chorioretinitis.

The radiologic examination (including that of the tetraplegic infant and 2 deaf ones) revealed no intracranial calcifications and the Cronqvist index was normal.

Psychologic examination revealed normal intellectual development in 21 cases. One infant (with tetraplegia) was mentally retarded. A second (the only infant small for gestational age) refused to cooperate but seemed to be mentally retarded. His retardation might have been due to constitutional factors.

Stillborn infants and infants who died perinatally. Examination of autopsy material from 38 of the 78 infants produced no evidence of CMV. In a further 13 cases congenital CMV infection could be excluded because of absence of CMV antibodies in the mother at delivery. One of the 2 cases of microcephaly in the material belonged to the latter group; in the second case CMV infection could not be excluded.

Control infants. Follow-up was possible in 51 of the 70 control infants selected. By the age of 6 months 16 (31 %) had acquired a postnatal CMV infection. The frequency of CMV excretors fell with increasing age (5/6 at 12 months, 0/4 at 30 months). Of 25 infants still virus-negative at 6 months and followed up further, 4 acquired a later CMV infection. In 4 of the 20 infected infants the excretion was heavy on some occasion. In the remaining cases



the excretion was medium or sparse. Three infants had recognizable signs of CMV (hepato- or splenomegaly). The control infants have developed normally without signs of hearing impairment.

#### Mothers of congenitally infected infants

Clinical symptoms. None of the 35 mothers of the congenitally infected infants had any symptoms characteristic of CMV infection (mononucleosis-like disease) in pregnancy.

Type of infection. Twelve of the mothers had a primary infection (CMV sero-conversion), 7 a secondary (CMV antibodies before conception) and 16 an unspecified type (no preconceptional sera; CMV IgG but no CMV IgM at first antenatal visit usually in gestational month  $\leq$  IV). In one woman with an unspecified infection the CMV CF titer rose suggesting secondary infection.

CMV CF and IIF-IgM. Twelve mothers had a CF-seroconversion. Three of the 7 women with a secondary infection and 1 of the 16 with an unspecified infection had a CF titer rise. After the seroconversions and titer rises the CF-titers in the mothers of the congenitally infected infants varied within the range of 64 and  $\geq$  512 (geometric mean  $\geq$  208).

IgM antibody activity was demonstrated in 11 of the 12 mothers with a primary infection; 10 achieved a titer between 64 and 512 (titer 64 considered as positive borderline); one showed a significant titer rise to a level below 64. In the only woman without demonstrable IgM after seroconversion, sera were obtained with an interval of 3 months. An IgM level of  $\geq$  64 was demonstrated for 3 - 6 months in 2 of 3 women followed up so long. Of the 7 women with secondary infection, none had any significant IgM activity (titers  $\leq$  16). In the group with an unspecified type of infection one had an IgM titer rise to below 64; (one had a stable titer of 40 from pregnancy month IV to delivery) and the remaining 14 women, titers of  $\leq$  16 until delivery.

Time of infection. The onset of infection could be specified by the seroconversion in 10 cases of primary infection: trimester I (2 cases), II (1 case), II-III (5 cases) and III (2 cases). In 3 secondary infections and 1 unspecified infection, the time of onset could be determined from the time of titer rise to trimester I, II-III, III and II, respectively.

Age, parity, occupation, nationality, seasonal variation. The women's ages varied from 20-35 years (Fig1). Fifteen (43 %) women were primigravidae, among them 7 (58 %) of the 12 with primary infection. None of the multiparae had more than 2 older children. Of the 12 mothers with a primary infection, 4 were hospital employees (one as children's nurse, the remaining 3 as doctor or assistant nurses at medical or geriatric clinics). Of the 23 mothers with secondary or unspecified infections, 4 were hospital employees. Twenty-three women had other occupations and 4 were housewives. All the mothers except 7 (3 of them with primary infection) were born Scandinavians. The onset of primary infection showed no seasonal variation.

#### Mothers of control infants

Only the mothers of the 51 control infants who were followed up will be described. All the 20 mothers of postnatally infected infants had stable CF titers within the range of 8 and 512 (geometric mean 119). Of the 31 mothers of infants who remained uninfected with CMV, 24 (77 %) were seropositive with stable CF titers during pregnancy ranging between 8 and 256 (geometric mean 51). No mother had a significant IgM activity (titers  $\leq 16$ ). Their ages varied between 18 and 36 years (Fig 1). Eighteen (34 %) of the women were primigravidae. Only few of the multiparae had more than 2 older infants. Thirteen women were hospital employees, 34 had other occupations and 4 were housewives. All but 6 were born Scandinavians.

#### Relation between the maternal type and time of infection and the infant's symptoms at birth and follow-up

The 7 infants with CMV symptoms at birth were born to mothers with primary (4 cases), secondary (1 case) or unknown (2 cases) types of infection (Table 1). The onset of infection could be established only for the primary type: trimester I (2 cases), II-III (1 case) and III (1 case).

Of the 4 infants with CMV sequelae, one (the child with tetraplegia) was infected due to a primary maternal infection in trimester I (CMV CF seroconversion on comparison of a serum obtained 9 months before conception and a post-conceptual serum, CMV IgM titer 320 in pregnancy month III). The 3 infants with isolated hearing impairment were infected after secondary (preconceptional antibodies) probably secondary (CMV IgG but no IgM in pregnancy month

IV, CF titer rise 2 months later) or unspecified type of infection (CMV IgG but no IgM in the middle of pregnancy month III, no subsequent titer rise) (Table 1). The child with a low birth weight and retarded development was born to a mother with a secondary type of infection.

## DISCUSSION

The frequency of congenital CMV infection found (0.5 %) bordered the lower limit of the range usually reported (0.5–1.5 %) (24), but was similar to that in a prospective virus isolation study in Denmark (0.4 %) (9). Low socioeconomic standard has been shown to be associated with a higher frequency of congenital CMV infection (28). The present material was unselected except for exclusion of some infants with severe malformations or severe respiratory distress. These symptoms are not typical for congenital CMV infection.

The impact of diagnostic methods on results is illustrated by a study of Melish et al (20), who found congenital infections in 1 %, most of them without sequelae, by virus isolation in unselected material and 0.1 %, representing 2 infants with sequelae, by virus isolation in selected infants with increased IgM (unspecific and specific) in cord serum. By indirect CMV-IgM test on cord serum both false positive and false negative reactions might be detected (27). The false positive tests are due to the reaction of IgM rheumatoid factor with viral IgG antibody in serum, RF is especially common in patients with congenital infections (23). Accordingly, a congenital infection other than CMV might give impression of CMV infection. Controls are necessary.

In the present study of 35 congenitally infected infants nearly all had no or only mild symptoms at birth; none died; none had neurological symptoms. Two (29 %) of the 7 infants with symptoms referable to the reticuloendothelial organs and 2 (8 %) of the 26 infants without symptoms at birth and with clear follow-up results developed neurologic sequelae attributable to CMV. Thus, 4 (12 %) of the 33 infants with assessable development have permanent symptoms due to CMV. At estimation of the general risk for CMV sequelae the total material of 8,367 individuals should be reduced with 5/38 to 33/38 (3 of 38 isolates in the pools not identified, 2 infants with no or not assessable follow-up results). Accordingly, the general risk should be 4 (0.06 %) in 7,266 individuals after a follow-up of 6 months–4 years (median 18 months). Only one of the 4 injured infants had mental retardation. As for minor sequelae and sequelae that have not yet appeared, the infants' development

will be finally judged at a planned 7 year review.

Our findings are in good agreement with earlier results in prospective virus isolation studies (9,10,18,20,29,30,31). These results are presented in Table 2. As shown in the table 6 (9 %) of 68 congenitally infected infants had symptoms at birth. One of the patients who had neurologic symptoms, 2 (40 %) of 5 who had reticuloendothelial symptoms and 2 (3 %) of 58 without symptoms at birth and assessable results at follow-up, that is, in all 4 (7 %) of 58, developed permanent neurologic sequelae attributable to CMV after a follow-up ranging from 6 months to 4 years.

Death due to congenital CMV infection is rare according to a review by Hanshaw et al (13). High frequencies ( $\geq 85$  %) of neurological sequelae in infants with CNS or reticuloendothelial symptoms at birth have been reported in the same review. It was not possible to verify this statement on the basis of the present prospective virus isolation studies or those in Table 2, since only few neonatally symptomatic infections were detected and followed up.

According to a review by Alford et al (8) neurological sequelae should also be common in neonatally asymptomatic infants: 18 % were shown to be severely handicapped by their neurologic deficit (microcephaly or IQ  $\geq 20$  points below normal); an additional number had minor IQ defects and deafness.

The serious prognosis reported in the reviews might be explained by a selection of more severely infected individuals because of the diagnostic methods used or by a longer follow-up.

In the 3 patients with isolated hearing impairment mental development was normal in all. Their power of concentration was good and they were mature for age and had a surprisingly good communication ability, probably a result of early training. It can be added that these patients constituted the main part of otoneurologic injuries detected in infants within the region and period.

The frequency of seropositive mothers varies between different countries and socioeconomic groups. In undeveloped countries it is close to 100 % (25), in England and USA it is 65-89 % (7) and in Sweden 72 % (5). Women with a low socioeconomic standard are more often seropositive than well-situated females in the same community (28). Accordingly, the possibility of contracting primary maternal infection (and congenital infection caused by the former) varies. In undeveloped countries practically all congenital infections must be due to secondary maternal infection. In Sweden the frequency has

been estimated to be up to two thirds (5). If primary and secondary maternal infections cause different morbidity in the offspring the pattern of congenital CMV disease should vary in different communities.

Interindividual variation e.g. of nutrition, cellular immunity and HLA (16) composition might also influence the morbidity.

The risk of transmission of a primary infection to the fetus has been estimated to be 50 % (5,22,31). Primary infections in trimester I and II are usually difficult to diagnose (lack of diagnostic material) but seem to play a dominant role in disease evident from birth (6). The most severely injured infant in the present study was infected by a primary maternal infection in trimester I. The remaining two maternal infections in trimester I or II have so far, after 12 and 18 months, not caused sequelae, although the infants were symptomatic at birth.

The significance of secondary maternal infection is difficult to estimate. The nature of the infection is easily overlooked because of lack of pre-conceptional sera or because of lack of diagnosis of the infant's disease (late start). Evidently only few of the common maternal secondary infections (7) are transmitted to the fetuses. In case of transmission the infants are presumed to be less virologically loaded and without sequelae (28). But judging from our findings, at least some infants born to mothers with a secondary infection had a massive virus excretion at birth and three cases of deafness could for more or less good reasons be attributed to secondary maternal infection. The literature contains 3 further possible cases, one of them fatal (6,14,33).

Present data are too scarce to warrant any general conclusions of the relative significance of primary and secondary maternal infections on the infant. Secondary infections may play a quantitatively important role.

The mothers of the congenitally infected infants did not differ markedly from the controls in age, parity, nationality or occupation. Primary maternal infection did not show any seasonal variation. The geometric mean of the CF titers was 208 in mothers of congenitally infected infants; mothers of postnatally infected and uninfected control infants had values of 119 and 51, respectively, probably indicating different virological stimulation. Mothers of uninfected control infants were generally multigravidae and older. Significant IgM activity (IIF titer  $\geq 64$ ) was only demonstrable in mothers with diagnosed primary infection.

The above-mentioned findings have implications on vaccination and abortion recommendation. The proven case of secondary maternal infection causing infant injury questions the effectiveness of vaccination. Prospective CMV IgM and IgG analyses and legal abortion after indicated primary infection would have prevented the birth of only one infant with injury (the infant with tetraplegia, mental retardation and deafness). At the same time an unknown number of uninfected or infected/non-injured infants would have been considered at risk.

The high frequency of postnatally acquired CMV infections clearly illustrates the difficulties to establish the diagnosis of congenital CMV infection beyond the first weeks of life.

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Table 1. Nine cases of congenital cytomegalovirus infection symptomatic at birth or later.  
Relation to maternal type and time of infection.

| Type and time of maternal infection | Hepatosplenomegaly and/or thrombocytopenic purpura at birth | Neurologic sequelae at follow-up       | Age in years at last control |
|-------------------------------------|-------------------------------------------------------------|----------------------------------------|------------------------------|
| <u>Primary</u>                      |                                                             |                                        |                              |
| Trimester I                         | +                                                           | Psychomotoric retardation and deafness | 2 1/2                        |
| " I                                 | +                                                           | -                                      | 1                            |
| " II-III                            | +                                                           | -                                      | 1 1/2                        |
| " III                               | +                                                           | -                                      | 2 1/2                        |
| <u>Secondary</u>                    |                                                             |                                        |                              |
| Trimester NS                        | +                                                           | Deaf                                   | 1 1/2                        |
| <u>Suggestive secondary</u>         |                                                             |                                        |                              |
| Trimester II                        | -                                                           | Deaf                                   | 2 1/2                        |
| <u>Unknown (secondary?)</u>         |                                                             |                                        |                              |
| Trimester NS                        | +                                                           | -                                      | 4                            |
| " NS                                | +                                                           | -                                      | 1/2                          |
| " NS                                | -                                                           | Deaf                                   | 2 1/2                        |

Table 2. Congenital cytomegalovirus infection detected by prospective virus isolation studies. Follow-up results.

| Source of data            | Infants infected/<br>studied | A t b i r t h                         |                                                            |                                                        | Follow-up<br>period   |
|---------------------------|------------------------------|---------------------------------------|------------------------------------------------------------|--------------------------------------------------------|-----------------------|
|                           |                              | Neurol. signs                         | Hepatosplenomegaly<br>and/or thrombo-<br>cytopenic purpura | No symptoms                                            |                       |
|                           |                              |                                       |                                                            |                                                        |                       |
|                           |                              |                                       |                                                            |                                                        |                       |
|                           |                              | Dead Neurol. Normal<br>sequel. devel. | Dead Neurol. Normal<br>sequel. devel.                      | Dead Neurol. Normal No<br>sequel. devel. follow-<br>up |                       |
| Stern, 1968               | 2/106                        |                                       | 1                                                          | 1                                                      | 6 -<br>18 months      |
| Birnbaum et al, 1969 (10) | 3/545                        |                                       | 2                                                          | 1                                                      | 12 -<br>18 months     |
| Stern et al, 1970 (30)    | 26/2,147                     | 1 <sup>1)</sup>                       |                                                            | (1) <sup>2)</sup> 24 <sup>3)</sup>                     | 8 months -<br>4 years |
| Kumar et al, 1973 (19)    |                              |                                       |                                                            |                                                        |                       |
| Melish et al, 1973 (21)   | 20/1,963                     |                                       | 1                                                          | 1 16 1                                                 | 2 years               |
| Stern et al, 1973 (32)    | 5/720                        |                                       |                                                            | 1 4                                                    | 1 year                |
| Andersen et al, 1979 (9)  | 12/3,060                     |                                       |                                                            | (2) <sup>4)</sup> 10                                   | 8 months -<br>2 years |
| Total                     | 68/8,541                     | 1                                     | 2 3                                                        | (1) <sup>2)</sup> 2(+2) <sup>4)</sup> 56 <sup>3)</sup> |                       |
| Present study             | 38 <sup>5)</sup> /8,367      |                                       | 2 5                                                        | 2(+1) <sup>4)</sup> 24                                 | 6 months -<br>4 years |

1. Intracerebral calcifications without clinical symptoms at birth. 2. Sudden death at 8 months of age.

3. Including one infant with transient neurologic disturbances. 4. Sequelae not attributable to CMV with certainty.

5. Three isolates not identified in pools of urine samples.

CONGENITAL CYTOMEGALOVIRUS INFECTION. ON THE RELATION BETWEEN TYPE AND TIME  
OF MATERNAL INFECTION AND INFANT'S SYMPTOMS

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## ABSTRACT.

Sera from the mothers of 45 live-born infants with congenital cytomegalovirus (CMV) infection and 4 fetuses with a CMV-background were analysed for CMV-IgG and -IgM. The purpose was to elucidate the relation between the maternal type and time of infection and the signs and symptoms of the offspring at abortion/birth and follow-up. Serological patterns compatible with primary maternal infection during trimesters I and II (6 cases), but also with secondary infection (in at least one case) were associated with infant sequelae or death. Asymptomatic infant infection was found after primary infection in trimesters II and II-III and after secondary infection. Virus was recovered only from one out of three fetuses legally aborted due to a primary infection in trimester I.

Attempts to demonstrate CMV IgM activity (marker of active infection) in early pregnancy (period of legal abortion) were successful in only five out of ten cases with infant sequelae or death. Symptoms at birth were prognostically bad, but the further course was sometimes uneventful even in children with neonatal signs of cerebral infection. A few children without initial symptoms developed sequelae (impairment of hearing).

## INTRODUCTION

Both primary and secondary (reactivated latent or re-infection) maternal cytomegalovirus (CMV) infections can be transmitted intrauterinely to the offspring. In highly immune populations the secondary type of infection is the main cause of congenital infection (24). In Sweden, where some 30 % of the women in fertile age are seronegative for CMV, the secondary infections might be responsible for up to two thirds of the 0.5 % congenital infections detected (6). To our knowledge neurologic sequelae in the infant have only been described after primary maternal infection within the first two trimesters (9,16,29) - with two possible exceptions (11,32). More information on the influence, if any, of secondary maternal infection on the development of the infant is urgent in order to ascertain whether vaccination of young women would be warranted. It is also important to ascertain to what extent serologic studies of maternal sera can detect fetuses at risk for severe congenital infection. The present study concerns a ten year material from Malmö and Stockholm, Sweden.

## MATERIAL AND METHODS

The study comprised four pregnant women and their legally aborted fetuses as well as 45 mothers and their congenitally infected live-born infants (19 girls, 26 boys). The infants excreting CMV were identified at diagnostic examination of neonatally symptomatic children or in previous and ongoing screening programs (1,3,5,6).

Virus isolation. Samples (tissues or urine) from the fetuses or infants were inoculated into cultures of local strains of human embryonal lung (HEL) fibroblasts, which were then observed for 4 - 6 weeks. CMV strains were identified by indirect immunofluorescence.

Definition of congenital CMV infection. This diagnosis was based on the demonstration of CMV excretion before the age of two weeks (usually within a few days of birth) or on CMV isolation from fetal organs.

Exclusion of other congenital infections. In cases of congenital CMV infection complicated by neurologic sequelae the possibility of a coexisting rubella, Toxoplasma or luetic infection was excluded by serologic tests.

Clinical examination. All the infants were examined in the neonatal period and then followed up by paediatricians. The terms "small for gestational age" (SGA), "appropriate for gestational age" (AGA), "preterm" and "term" refer to growth charts based on studies of a Swedish infant material (12).

Sera. Maternal sera were collected during pregnancy and at delivery. In 13 cases preconceptional sera, obtained up to 3 years before pregnancy, were available. The sera were stored at  $-20^{\circ}\text{C}$ .

Serological tests. Maternal sera were examined for CMV antibody activity with complement fixation (CF) (25), for CMV IgG with an indirect enzyme-linked immunosorbent assay (ELISA) (5,31) and for CMV IgM with an indirect immunofluorescence test (IIF) (2). In a few cases further checks for IgM antibody activity were performed with indirect ELISA and the enzyme-labelled antigen (ELA) technique (23). Sera positive in the indirect IgM tests were tested for rheumatic factors (Latex-RF-Reagenz, Behringwerke, Germany) (26). Independent of the result of the RF test a corroborative IgM test was (with few exceptions) performed after absorption with aggregated human IgG (13) and/or after serum fractionation in a sucrose density gradient (100,000 g for 18 hours). The CF antigen was an alkaline glycine extract (8) of cells infected with CMV AD 169. The same antigen was used in the ELISA test carried out in Malmö, whereas CMV AD 169 infected HEL cell nuclei were used in Stockholm (22). Antigen for ELA was kindly furnished by Professor H. Schmitz, Western Germany.

Background to the classification of maternal infection. Following a primary CMV infection virus specific antibodies of IgM, IgG (and IgA) class appear in serum. IgM antibodies appear at a significant level ( $>64$  by IIF) within the first two weeks and persist for 1 - 6 months (10 patients). A significant level of CMV IgM was occasionally found in CMV IgG positive pregnant women with non-CMV-excreting newborns (2 of 500) as well as in blood donors (1 of 200).

IgG, as measured by ELISA (and CF), appears at the same time or some weeks after the specific IgM in cases of primary infection. The ELISA IgG and CF activities persist for years or the rest of life. It has, however, been reported that CF antibodies might disappear after CMV infection both in infants (2,15) and adults (30). But, in those studies the CF antigen used was less potent.

Secondary CMV infection may or may not influence the serum antibody level. In most gravidæ with secondary infection no CF titer rise is demonstrable (20). IgM activity might occur, as described in verified cases of secondary CMV infection in non-pregnant materials (17,19).

## RESULTS

Clinical and laboratory findings of mothers and live-born infants are presented in Table 1 with the exception of nine cases of asymptomatic infant infection and unknown type of maternal infection. Data on all 45 live-born children are summarized in Table 2.

### Live-born infants

#### Symptoms and signs compatible with CMV infection in the neonatal period

The central nervous system (CNS) was involved in five infants (Cases 1,4,7, 13,34): four had intracerebral calcifications, two of them also chorioretinitis, and one had muscular hypotonia. None was microcephalic. All five had also general symptoms (see below).

General symptoms of CMV infection, such as hepatomegaly, icterus, splenomegaly and/or thrombocytopenic purpura, but no signs of involvement of the CNS, were found in ten infants. One (Case 29) of them had a fulminant cytomegalic inclusion disease (CID).

No symptoms attributable to the congenital infection appeared in thirty infants.

#### Symptoms at follow-up

Motor/psychomotor disability was evident in five infants. In three of them (Cases 1,2,7) it was combined with impaired hearing and, in one, also with impaired vision and a head circumference bordering the lower limit of the normal range ( $-2SD$ ). All three had neurologic or general symptoms of CMV infection at birth. The fourth infant (Case 4) was born in gestational week 38 with a birth weight of 1930 g and with severe general symptoms of CMV infection, hypotonia and absent reflexes. At one year of age he was small for age and motorically retarded, but mentally normal and had a normal audiogram.

### III:6

Another infant (Case 32) was born at the gestational age of 30 weeks to a mother in whom an intrauterine device was still in position. He had a low birth weight (1630 g) but no characteristic neonatal symptoms of CMV infection. He has repeatedly had debilitating infections. At 1 1/2 year of age he was psychomotorically retarded. His audiogram was normal.

Severe neurologic impairment of hearing is the only permanent symptom detected in five infants (Cases 3,5,25,27,35). Three of them had general symptoms of CMV infection at birth and two were asymptomatic. At three months of age three infants had a reversible impairment of the traction response with maximal head lag. At least two of the children have been able to communicate in a deaf-and-dumb language since early age.

Normal development. Thirty-one children are normally developed at the age of 1/2 - 7 years (median 1 1/2 year). Two of them (Cases 13,34) were born with signs of CNS involvement (including paraventricular calcifications) and general symptoms of CMV infection. Four infants (Cases 9,18,31,36) had only general CMV symptoms at birth and 25 were asymptomatic. Audiograms were recorded in 23 children (including 5 of those initially symptomatic) aged 1/2 - 6 years (median 1 year). The remaining infants have passed general hearing control. All the infants tested had normal hearing.

Four infants were not followed up: two of them died from CID and VOC, respectively, and two moved out of town.

#### Maternal infections

Three women had characteristic signs of their CMV infection (mononucleosis-like disease). The symptomatic cases were found among those 20 women who had a serological pattern compatible with primary infection. In 17 of these women seroconversion occurred during pregnancy or between pre-pregnancy and preparation of first postconceptional serum. A high CMV IgM level in this postconceptional sample made a primary infection during early pregnancy probable in these as well as in three women without preconceptional serum. Secondary infection was proved in 5 cases (CMV antibodies before conception). In 20 women differentiation between primary infection in the first part of pregnancy and secondary infection at any time was not possible (IgG+IgM- in first postconceptional serum). In 5 of these cases of unspecified infection subsequent CMV IgG and/or IgM titer rise suggested a secondary infection.

A significant CMV IgM level (IIF-titer  $\geq 64$ ) was demonstrable in 14 of 16

mothers with a primary infection diagnosed by seroconversion; in a further case it rose significantly to  $<64$ ; the IgM negative woman was tested with an interval of 3 months. All the five mothers with proven secondary infection lacked IgM activity.

#### Relation between type and time of maternal infection and infants symptoms.

In the two fatal cases the serological analyses of maternal sera did not allow a firm diagnosis of the type of maternal infection. However, in both cases the antibody pattern suggested secondary infection (see above). In one (Case 29: fulminant CID) of the cases also additional methods (IIF against the strain isolated from the infant, ELISA on IgM fractions and whole sera, and ELA) were used in examination of the first serum for IgM. Still only traces of CMV IgM were demonstrable. In the second case (Case 30: VOC) the maternal infection seems to have occurred after organogenesis.

The five children with motor/psychomotor disability were born to mothers with serologic signs compatible with primary infection in trimester I (Cases 1,2,4) or II (Case 7). In the fifth case (Case 32) no information on the type of infection was obtained. Mothers of infants with isolated hearing impairment had either a primary infection in trimester I (Cases 3,5) or a secondary (Case 25) or unclassified infection (Cases 27,35). In Case 27 the infection might have been secondary as suggested by titer rise.

Neonatally symptomatic infection without apparent sequelae was observed after primary maternal infection in trimesters II-III (Cases 9,13) and III (Case 18) and after unspecified type of infection (Cases 31, 34, 36). Infant infection asymptomatic at birth and follow-up occurred after primary infection in trimesters II (2 cases), II-III (5 cases), III (2 cases) and I-III (1 case). It was also seen after secondary (4 cases) and unclassified infections (11 cases).

#### Fetuses and their mothers

Three women with a serological pattern compatible with primary CMV infection in trimester I underwent legal abortion. Two of them had an uncharacteristic disease, either a protracted respiratory infection or headache, myalgia and gastroenteritis. They were both seronegative 2 years before conception. Serologic follow-up, performed at their own request, in gestational month III showed sero-conversion and IgM response. No CMV was recovered from amniotic fluid or organs from the fetuses. The third woman had a common cold in gesta-



tional month III followed two weeks later by thrombocytopenic purpura and internal and external haemorrhages. Because of the microscopic appearance of the sternal punctate the disease was first misdiagnosed as leukemia. However, IIF IgM titer 128 and virus isolation indicated CMV infection. CMV was also recovered from several fetal organs but not from the brain.

In a fourth case legal abortion was performed for social reasons. The fetus, 19 weeks old, was icteric and had hepatosplenomegaly and ascites. CMV was isolated from the lungs (the only material obtained). Histopathologic examination revealed CMV inclusions in the placenta and liver and inflammatory loci in the kidneys and brain. The type and time (<19 gestational weeks) of the maternal infection are unknown.

#### DISCUSSION

Well defined criteria for the diagnosis of congenital and maternal CMV infection are essential. In this study the diagnosis of congenital infection was based on recovery of CMV from fetal organs or from urine of live-born infants within two weeks - in most cases within a few days - of birth. At this age a natively infected child has not started to excrete CMV (20). It may obviously be difficult to distinguish between primary and secondary maternal infections. Only sero-conversion shown to occur during pregnancy and CMV-antibodies in preconceptional sera have been considered to prove primary and secondary infection, respectively, but also significant IgM activity has been considered to indicate primary infection.

There might also have been injurious factors other than the congenital CMV infection. Thus, the role of CMV is unclear in one of the infants with psychomotor retardation (intrauterine device, low birth weight, repeated infections) and in the infant with VOC. Ototoxic drugs were given to two children (Cases 7,25) with neonatal disease. It is, however, unlikely that the short medication had contributed to the hearing impairment.

The development of congenitally infected infants cannot be definitely assessed before the child has been followed for many years (7). Because of the low age of many of the infants in the present study, 6 month - 8 years (median 1 1/2 year), some of the hitherto asymptomatic children might still develop symptoms.

The results of our study corroborate that CMV symptoms at birth are signs of bad prognosis (18). In a given case the development may, however, be more favourable than expected. Of the five infants with neonatal involvement of

the CNS, two have hitherto developed normally. Of eight infants with general CMV symptoms at birth, four appeared normal at the age of 1 - 6 years. After a follow-up of 1/2 - 7 years (median 1 1/2 year) only two of 28 initially asymptomatic infants have obvious sequelae (hearing impairment).

The estimated risk of permanent neurologic sequelae after a CMV infection with central nervous or peripheral symptoms in the neonatal period is reportedly very high, 85 and 80 %, respectively (10). Estimation of the risk figure for patients with neonatally asymptomatic infection requires prospective studies using optimal methods for detection of the infection. At present virus isolation is the most sensitive test. Only few of the neonatally asymptomatic infections detected by this test are followed by sequelae (14,15).

According to our interpretation of the serologic data it is clear that a primary maternal infection, especially in trimester I, but also in trimester II, is a serious, though not unexceptional, threat to the child. This finding is in agreement with the limited data in the literature (16,29). However, it is also clear that a maternal infection of secondary type may result in a congenital infection compatible with CMV disease at birth and with neurologic sequelae of the type seen after primary CMV infection (Case 25) (4). Some earlier data have been suggestive in this respect: Yeager et al (32) reported on two siblings born one year apart and both congenitally infected with CMV. The second child developed neurologic disease - seizures - at seven months of age. Hayes et al (11) described an immunodepressed woman with preconceptional antibodies to CMV, who gave birth to a neonatally asymptomatic infant excreting CMV. Later the infant developed severe brain damage.

It cannot be excluded that secondary maternal CMV infection might be more than an exceptional cause of infant disease. In our material there may be two or even three more cases (Cases 27,29,35) concealed among those in which the maternal infection could not be characterized in spite of samples obtained in early pregnancy. If preconceptional sera are not available the diagnosis of a secondary maternal infection is evidently more easily missed than that of a primary infection. The proportion of congenital CMV disease caused by secondary maternal infection can be estimated only from combined prospective studies of mothers and infants. Such studies are in progress (5,6,28). Access to banks of preconceptional sera is necessary.

In a prospective study in Sweden (6) the frequency of CMV excretors among newborns was found to be 0.5 %. Sequelae, due to congenital CMV, were noted in 0.06 % of the total material after a follow-up period of 1/2 - 4 years.

Judging from such figures, every year about 60 out of 100,000 newborn Swedish infants will have more or less severe congenital CMV disease. Preventive measures are desirable. Until we have better knowledge on the effect of vaccination against CMV other means must be considered.

Most maternal CMV infections - even the primary ones - run a silent course. However, serologic analysis might detect at least the primary infections. In the present material prospective CMV IgM and IgG analyses performed during early pregnancy would have predicted congenital CMV with sequelae or fatal outcome in five of ten live-born infants. However, an unknown number of unaffected fetuses - infected or not - would have been regarded as being at risk (compare the two cases of aborted non-infected fetuses). No figures for the risk of congenital CMV disease after primary and secondary maternal infections in different trimesters are yet available. They will have to be assessed from large prospective studies. At diagnostic examination the maternal serologic pattern might be ambiguous and create practical difficulties (21,27).

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Table 1. Thirty-six case reports on maternal and congenital CMV infection.

| M O T H E R                           |             |             |                  |                                    |                                                                       |
|---------------------------------------|-------------|-------------|------------------|------------------------------------|-----------------------------------------------------------------------|
|                                       |             |             |                  |                                    | Serologic data                                                        |
| Type and time<br>of CMV inf.          | Case number | Age (years) | No. of pregnancy | Mononucleosis-<br>like CMV disease | Preconceptionally<br>Month I II III IV V VI VII VIII IX X<br>Delivery |
| PRIMARY TYPE                          |             |             |                  |                                    |                                                                       |
| Trimester I                           |             |             |                  |                                    |                                                                       |
| Case 1                                | 29          | 3           |                  |                                    | — ●                                                                   |
| " 2*                                  | 24          | 3           |                  |                                    | ○ — ●                                                                 |
| " 3                                   | 21          | 2           |                  |                                    | — ●                                                                   |
| " 4                                   | 24          | 2           |                  |                                    | ○ — ●                                                                 |
| " 5                                   | 27          | 2           |                  |                                    | ○ — ●                                                                 |
| Trimester II                          |             |             |                  |                                    |                                                                       |
| Case 6                                | 32          | 2           |                  |                                    | — ○ — ●                                                               |
| " 7                                   | 25          | 2           |                  |                                    | — ●                                                                   |
| " 8*                                  | 20          | 1           |                  |                                    | ○ — ○ — ●                                                             |
| Trimester II-III                      |             |             |                  |                                    |                                                                       |
| Case 9*                               | 26          | 1           |                  |                                    | ○ ○ — ○ — ●                                                           |
| " 10                                  | 32          | 1           |                  |                                    | ○ ○ — ○ — ●                                                           |
| " 11*                                 | 24          | 1           |                  |                                    | ○ — ○ — ●                                                             |
| " 12*                                 | 35          | 1           |                  |                                    | ○ — ○ — ●                                                             |
| " 13                                  | 29          | 1           |                  |                                    | ○ — ○ — ●                                                             |
| " 14*                                 | 32          | 1           |                  |                                    | ○ — ○ — ●                                                             |
| " 15                                  | 25          | 1           |                  |                                    | ○ — ○ — ●                                                             |
| Trimester III                         |             |             |                  |                                    |                                                                       |
| Case 16                               | 33          | 2           |                  | x                                  | ○ — — — — —                                                           |
| " 17                                  | 41          | 8           |                  | x                                  | — — — — —                                                             |
| " 18*                                 | 26          | 1           |                  |                                    | ○ — — — — —                                                           |
| " 19                                  | 30          | 2           |                  | x                                  | ○ — — — — —                                                           |
| Trimester I-III                       |             |             |                  |                                    |                                                                       |
| Case 20*                              | 29          | 2           |                  |                                    | ○ — — — — —                                                           |
| SECONDARY TYPE                        |             |             |                  |                                    |                                                                       |
| Case 21*                              | 33          | 2           |                  |                                    | ■ — — — — —                                                           |
| " 22*                                 | 26          | 3           |                  |                                    | ■ — — — — —                                                           |
| " 23*                                 | 24          | 4           |                  |                                    | ■ — — — — —                                                           |
| " 24*                                 | 34          | 3           |                  |                                    | ■ — — — — —                                                           |
| " 25*                                 | 27          | 3           |                  |                                    | ■ — — — — —                                                           |
| UNSPECIFIED TYPE                      |             |             |                  |                                    |                                                                       |
| a) with titer rise (sec.inf.probable) |             |             |                  |                                    |                                                                       |
| Case 26                               | 19          | 2           |                  |                                    | ■ — — — — —                                                           |
| " 27*                                 | 24          | 3           |                  |                                    | — — — — —                                                             |
| " 28*                                 | 22          | 1           |                  |                                    | — — — — —                                                             |
| " 29                                  | 33          | 2           |                  |                                    | — — — — —                                                             |
| " 30                                  | 19          | 1           |                  |                                    | — — — — —                                                             |
| b) without titer rise                 |             |             |                  |                                    |                                                                       |
| Case 31                               | 20          | 1           |                  |                                    | ■ — — — — —                                                           |
| " 32                                  | 27          | 2           |                  |                                    | — — — — —                                                             |
| " 33                                  | 20          | 2           |                  |                                    | — — — — —                                                             |
| " 34                                  | 23          | 1           |                  |                                    | — — — — —                                                             |
| " 35*                                 | 26          | 2           |                  |                                    | — — — — —                                                             |
| " 36*                                 | 22          | 1           |                  |                                    | — — — — —                                                             |

For references and signs see page III:16

## I N F A N T

| Case number | Sex | SGA, AGA <sup>1)</sup> | Preterm <sup>1)</sup><br>Term | CNS signs | General symptoms<br>of CMV infection | Symptoms at follow-up |                                                  |                              |
|-------------|-----|------------------------|-------------------------------|-----------|--------------------------------------|-----------------------|--------------------------------------------------|------------------------------|
|             |     |                        |                               |           |                                      | Age (years)           | CNS sequelae<br>with/without<br>hearing impairm. | Isolated<br>hearing impairm. |
| Case 1      | F   | AGA                    | T                             | x         | x                                    | 2                     | x                                                |                              |
| " 2*        | F   | AGA                    | P                             |           | x                                    | 1 1/2                 | x                                                |                              |
| " 3         | F   | AGA                    | T                             |           | x                                    | 8                     |                                                  |                              |
| " 4         | M   | SGA                    | T                             | x         | x                                    | 1                     | x                                                | x                            |
| " 5         | F   | AGA                    | P                             |           | x                                    | 1/2                   |                                                  | x                            |
| Case 6      | M   | AGA                    | T                             |           |                                      | 1 1/2                 |                                                  |                              |
| " 7         | M   | SGA                    | T                             | x         | x                                    | 8                     | x                                                |                              |
| " 8*        | M   | AGA                    | T                             |           |                                      | 1                     |                                                  |                              |
| Case 9*     | F   | AGA                    | T                             |           | x                                    | 1                     |                                                  |                              |
| " 10        | M   | AGA                    | T                             |           |                                      | 1/2                   |                                                  |                              |
| " 11*       | M   | AGA                    | T                             |           |                                      | 1 1/2                 |                                                  |                              |
| " 12*       | F   | AGA                    | T                             |           |                                      | 1                     |                                                  |                              |
| " 13        | M   | AGA                    | T                             | x         | x                                    | 2 1/2                 |                                                  |                              |
| " 14*       | F   | AGA                    | T                             |           |                                      | 1 1/2                 |                                                  |                              |
| " 15        | M   | AGA                    | P                             |           |                                      | 4                     |                                                  |                              |
| Case 16     | M   | AGA                    | T                             |           |                                      | < 1/2                 |                                                  |                              |
| " 17        | M   | AGA                    | T                             |           |                                      | 7                     |                                                  |                              |
| " 18*       | F   | AGA                    | T                             |           | x                                    | 2 1/2                 |                                                  |                              |
| " 19        | F   | AGA                    | P                             |           |                                      | 4                     |                                                  |                              |
| Case 20*    | M   | AGA                    | T                             |           |                                      | 1/2                   |                                                  |                              |
| Case 21*    | M   | AGA                    | T                             |           |                                      | 2 1/2                 |                                                  |                              |
| " 22*       | F   | AGA                    | T                             |           |                                      | 2 1/2                 |                                                  |                              |
| " 23*       | M   | SGA                    | T                             |           |                                      | 1 1/2                 |                                                  |                              |
| " 24*       | F   | AGA                    | T                             |           |                                      | 1                     |                                                  |                              |
| " 25*       | M   | AGA                    | T                             |           | x                                    | 1/2                   |                                                  | x                            |
| Case 26     | F   | SGA                    | P                             |           |                                      | 2                     |                                                  |                              |
| " 27*       | F   | AGA                    | T                             |           |                                      | 1 1/2                 |                                                  | x                            |
| " 28*       | M   | AGA                    | T                             |           |                                      | 1/2                   |                                                  |                              |
| " 29        | M   | AGA                    | T                             |           | x                                    | < 1/2                 | † CID                                            |                              |
| " 30        | M   | AGA                    | T                             |           |                                      | < 1/2                 | † (VOC)                                          |                              |
| Case 31     | M   | AGA                    | P                             |           | x                                    | 6                     |                                                  |                              |
| " 32        | M   | AGA                    | P                             |           |                                      | 1 1/2                 | (x)                                              |                              |
| " 33        | F   | SGA                    | T                             |           | x                                    | < 1/2                 |                                                  |                              |
| " 34        | M   | AGA                    | P                             | x         | x                                    | 1 1/2                 |                                                  |                              |
| " 35*       | F   | AGA                    | T                             |           |                                      | 1 1/2                 |                                                  | x                            |
| " 36*       | M   | AGA                    | T                             |           | x                                    | 2 1/2                 |                                                  |                              |

Table 1

1) Defined under "Material and Methods"

2) Cord serum

Possible time of maternal infection: - - - - -

Assumed time of maternal infection: \_\_\_\_\_

o Negative CMV-IgM and -IgG tests

● Significantly positive CMV-IgM test

▲ Significant rise of CMV-IgG and/or -IgM activity

■ Positive, stationary CMV-IgG test without significant -IgM rise/activity

\* From the prospective study (6)

Table 2. Infants congenitally infected with CMV: Signs and symptoms at birth and clinical development.

| A t b i r t h                                                   | A t f o l l o w - u p |             |                                          |             |                         |                 |                 |                     |                        |             |
|-----------------------------------------------------------------|-----------------------|-------------|------------------------------------------|-------------|-------------------------|-----------------|-----------------|---------------------|------------------------|-------------|
|                                                                 | Dead                  |             | CNS sequelae with/without hear. impairm. |             | Isolated hear. impairm. |                 | Hitherto normal |                     | Followed up < 1/2 year |             |
|                                                                 | No. pat               | Age (weeks) | No. pat                                  | Age (years) | No. pat                 | Age (years)     | No. pat         | Age (years)         | No. pat                | Age (years) |
| Signs and symptoms                                              |                       |             |                                          |             |                         |                 |                 |                     |                        |             |
| <u>CNS signs/symptoms combined with general symptoms</u>        | 5                     |             | 3                                        | 1, 2, 8     |                         |                 | 2               | 1 1/2, 2 1/2        |                        |             |
| <u>General symptoms</u>                                         | 10                    | 1 (CID)     | 1                                        | 1 1/2       | 3                       | 1 1/2, 1 1/2, 8 | 4               | 1, 2 1/2, 2 1/2, 6  | 1                      | < 1/2       |
| Hepatospleno-<br>megaly and/or<br>thrombocyto-<br>penic purpura |                       |             |                                          |             |                         |                 |                 |                     |                        |             |
| <u>No symptoms referable to the CMV infection</u>               | 30                    | 1 (VOC)     | (1)                                      | 1 1/2       | 2                       | 1 1/2, 1 1/2    | 25              | 1/2 - 7 (med 1 1/2) | 1                      | < 1/2       |
| Total                                                           | 45                    | 2           | 4(+1)                                    |             | 5                       |                 | 31              |                     | 2                      |             |



## RISK OF CYTOMEGALOVIRUS INFECTION IN NURSES AND CONGENITAL INFECTION IN THEIR OFFSPRING

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**ABSTRACT.** Ahlfors, K., Ivarsson, S.-A., Johnson, T. (†) and Renmarker, K. (Department of Clinical Virology and Department of Paediatrics, University of Lund, Malmö General Hospital, Malmö, and the Department of Personnel Health Care, Malmö, Sweden). Risk of cytomegalovirus infection in nurses and congenital infection in their offspring. *Acta Paediatr Scand*, 70: 819, 1981.—The risk of contracting cytomegalovirus (CMV) infection in nursing of infants and of congenital CMV infection in infants born to such nursing personnel were investigated. The investigation comprised 292 women working in paediatric clinics or day nurseries and a control group of 163 women who had no professional contact with infants. Among the women younger than 25, those who had tended infants for more than six months were significantly ( $p < 0.001$ ) more often seropositive for CMV than were those—mainly student nurses—with less than six months' infant nursing service, but not more often than control women. At ages above 25 there was no intergroup difference in regard to CMV tests. Seroconversion was rare and there was no demonstrable difference between the groups. In a separate study the occupation of 36 mothers of infants with congenital CMV infection was investigated. Compared to a control group no overrepresentation of nurses was found. All six congenitally infected infants born to nurses developed normally.

**KEY WORDS:** Cytomegalovirus, congenital, serology, nurses

Congenital cytomegalovirus (CMV) infection is a well known cause of neurologic disturbances. So far, analysed cases of such disorders in infants have been shown to be the result of primary maternal CMV infection contracted within the first six months of pregnancy (1, 2). However, secondary maternal infection (reactivated latent or re-infection) cannot be excluded as a risk to the fetus (3, 4).

In Malmö (5, 6, 7) we found CMV in the urine of 25 to 50% of infants aged 3 to 12 months. In most cases this was due to perinatal or neonatally acquired infections. We now wished to explore the possibilities of increased risk of CMV infection among infant-nursing personnel and of congenital CMV infection in children born to women in such occupations.

We therefore studied the CMV antibody status in female graduate and student nurses tending infants and in a control group of women. Those who were seronegative were followed up for evidence of primary CMV in-

fection. Conversely, we registered the occupations of the mothers of infants in whom congenital CMV infection had been diagnosed in the Malmö region during the past decade. Comparison was made with mothers of infants without congenital CMV infection. The combined frequency of primary and secondary maternal CMV infections transmitted to the fetuses could thus be studied according to maternal occupation.

### MATERIALS AND METHODS

*Studied women.* Graduate or trainee nurses in Malmö were assigned to one or other of two *risk groups* according to the extent of their occupational contact with infants. Both groups had professionally nursed infants aged 3 to 12 months. Some women had in addition nursed premature and sick newborns. Only few of these newborns were congenitally infected.

1. Risk group I comprised 162 nurses, including some students, all of whom had worked for at least six months in paediatric clinics or day nurseries.

2. Risk group II contained 130 women who had been engaged in the care of infants for less than six months.



Table 1. Risk group I according to age, duration of infant tending and serologic status

| Age (yrs)                                  | Duration (yrs) of infant tending |       |       |       |
|--------------------------------------------|----------------------------------|-------|-------|-------|
|                                            | ½-1                              | 2-4   | 5-9   | ≥10   |
| <i>CMV-CF positives: individuals/total</i> |                                  |       |       |       |
| ≤24                                        | 10/12                            | 13/21 | 2/4   |       |
| 25-29                                      | 4/4                              | 22/29 | 16/20 | 1/1   |
| 30-34                                      | 1/2                              | 6/6   | 7/8   | 6/7   |
| ≥35                                        | 0/1                              | 4/4   | 7/9   | 29/34 |

Some were trained nurses, but most were student nurses taking a six-week course in paediatric nursing.

3. The controls were 163 women who had not worked in the health services. Most of the younger women were cleaners and most of the older women were office workers.

The occupational study comprised the mothers of 36 infants congenitally infected with CMV (23 of them from a prospective study) and the mothers of 36 infants without congenital CMV infection (all mothers from the prospective study). Most of the mothers are included in other reports (4, 7, 8). The occupational information was obtained from the mothers themselves or from the case records.

Possible differences in social status were not taken into account in any of the two studies.

*Sera.* The women entered the serological study in the period 1978-1980. Sera from graduate or student nurses were obtained within six weeks of commencing infant care. According to the results of complement fixation (CF) tests, the women were classified as seropositive or seronegative. In seronegative student nurses the test was repeated three months later (more than 6 weeks after completion of the paediatric course). Seronegative graduate nurses were retested after 3, 6 and 9 months, and thereafter at yearly intervals. The seronegative controls were tested once a year. The study did not include attempts to diagnose secondary infections. Sera were stored at -20°.

*Laboratory tests.* All serum samples were tested without delay for complement fixing antibody to CMV anti-

gen. An alkaline glycine buffer extract of CMV-AD-169-infected human embryonal lung fibroblasts was used as antigen (9). The CF test was performed according to the micromethod described by Sever (10). CF titer ≥8 was considered a positive reaction and a fourfold or more increase as significant change. In case of CF-seroconversion, considered as a probable sign of primary infection (authors' criteria for primary and secondary CMV infections, see ref. 4), all sera from the same individual were re-tested in the same CF run. The sera were also examined with an indirect immunofluorescence (IIF) test for CMV-specific IgM antibody activity (11). IgM titer 64 was considered a borderline reaction and ≥128 a positive reaction indicating an active, probably primary CMV infection (4). As rheumatoid factors (RF) could cause false positive reactions (12), IgM-positive sera were checked with an RF test (Latex-RF-Reagenz, Behringwerke, Germany).

*Statistical treatment.* In comparing two relative frequencies we used the binomial *t*-test with Yates' correction. For comparisons of three or more frequencies we used the  $\chi^2$  test or the *F*-test for several ratios; when expected frequencies were less than 5, a non-parametric test (Fisher's exact test) was used.

## RESULTS

The duration of occupational contact with infants increased with age in risk group I (Table 1). The frequency of seropositive nurses increased with age, but it did not vary significantly with the duration of exposure to sick infants.

The percentages of seropositive women according to age in the two risk groups and in the controls are shown in Table 2. In each group the frequency of seropositives increased with age. When the totals of the various groups were compared, the differences in seropositivity were only weakly significant ( $p < 0.05$ ). Intergroup comparisons at different age levels, however, showed that at age <25 the frequency of seropositive reactions was significantly lower ( $p < 0.001$ ) in risk group II than in the other two groups. In the older women there was no such intergroup difference.

In the cumulative follow-up period of 31 years in risk group I (29 of 34 seronegative women) there were two seroconversions. In risk group II there were no seroconversions (total follow-up 16 years in 31 of 45 seronegative women). No student nurse among those followed up (3 of 7 in group I and 19 of 29 in group II) contracted CMV infection during

Table 2. Age and group distribution of seropositive women

| Age (yrs)                  | Risk group I (≥6 months' infant tending) | Risk group II (<6 months' infant tending) | Controls (no infant tending) |
|----------------------------|------------------------------------------|-------------------------------------------|------------------------------|
| <i>Seropositives/total</i> |                                          |                                           |                              |
| ≤24                        | 25/37 (68%)                              | 20/45 (44%)                               | 26/45 (58%)                  |
| 25-29                      | 43/54 (80%)                              | 24/33 (73%)                               | 22/36 (61%)                  |
| 30-34                      | 20/23 (87%)                              | 17/24 (71%)                               | 17/27 (63%)                  |
| ≥35                        | 40/48 (83%)                              | 24/28 (86%)                               | 46/55 (84%)                  |

paediatric training. Of the 52 controls in the follow-up study, only one showed seroconversion in a total follow-up of 32 years. In relation to the totals of "seronegative woman years", therefore, the respective ratios of seroconversion in the three groups were 2:31, 0:16 and 1:32. These ratios are not statistically different (Fisher's exact test).

Of the two group I nurses with seroconversion, one had subclinical CMV infection after working for two years at the paediatric clinic. She was then 20 years old, nullipara and nulligravida. The other infected nurse had conceived shortly after starting work at the paediatric clinic. Previously she had worked with infants for one year and with adult patients for two years. This was her first pregnancy and she was 26 years old. The CMV infection, which occurred between mens V-IX, was subclinical. Transmission of CMV to her infant was demonstrated by recovery of CMV from its urine at birth. The control (office worker) with seroconversion was 30 years old and had two children, both of whom attended a day nursery. She was not pregnant and the infection was subclinical. The CF titers in these three women rose from <8 to 256 or  $\geq 512$ .

The IgM titers in the two women tested at three-monthly intervals (group I) rose from <16 to 64 and 256, respectively. In the third case (office worker), with serum sampled at yearly intervals, no specific IgM antibodies were detected.

The occupations of the 36 mothers of infants with congenital CMV infection included hospital nursing in six cases; two of these six nursed infants. One mother was a doctor (not paediatrician); two were private childminders and one was a dental nurse. The remaining 26 mothers had no professional contact with children; they included 18 women with diverse occupations, 3 students and 5 housewives. Of the 36 control women, six were employed in hospital nursing, one of them in infant nursing. Two mothers were kindergarten teachers; one mother was a child-minder. The 27 other women included 22 individuals with diverse

professions, 1 student and four housewives. There was no statistical difference (Fisher's exact test) in frequency of hospital infant-nursing personnel in the group with congenitally infected infants (two of 36) and in the control group (one of 36).

The age of the six hospital nurses among the mothers with CMV-infected infants ranged from 20 to 34 years (mean 28). The corresponding figures for the other 30 women in this group were 20-41 (mean 26). Three of the six and 15 of the 30 CMV-infected children had older siblings. In the control mothers the age range was 18-38 years (mean 27) and 20 (56%) of the infants had older siblings.

In 15 of the 36 infants with congenital CMV infection, the maternal infection was assumed to be of primary type (CF-seroconversion and/or IgM-titer  $\geq 128$ ) (4). These 15 mothers comprised the doctor, two of the hospital nurses (one caring for infants), the dental nurse, two child-minders, one student, two housewives and six women with varying occupations.

All the six congenitally CMV-infected infants of hospital nursing personnel developed normally. They are now aged nine months to four years (mean 18 months).

## DISCUSSION

As shown in a separate study enzyme-linked immunosorbent assay (ELISA) instead of CF would not have changed the results significantly (8).

The frequency of positive serum tests for CMV increased with the women's age. This trend was similar in risk groups I and II and in the control group (83-86% after age 35). In a separate study of about 4500 pregnant women representing four-fifths of all gravidae in Malmö during a two-year period (mothers of infants studied prospectively for congenital CMV-infection) (8), 79% of those older than 35 were seropositive for CMV.

Among the women younger than 25, those from risk group I (who had tended infants for more than 6 months), from the control group,

or from the above-mentioned series of 4500 gravidae (8) were seropositive (58–71%) significantly more often than the women in risk group II (44%). Group II consisted mainly of student nurses, but contained some graduates with only brief service in paediatrics. The young women from risk group II thus seem to have been less exposed to CMV than the other groups of women.

Even after many years of nursing infants, some women remained seronegative. This frequency was the same as for non-nurses of corresponding age.

The frequency per year of conversion from negative to positive serum test for CMV was similar in the two risk groups and in the controls (0–6.5%). In the separate series of 4500 pregnant women (8) the corresponding frequency was 2.4% (14 seroconversions in 595 "seronegative woman years").

The present annual birth rate in the city of Malmö (c. 225 000 inhabitants) is approximately 2500. The annual frequency of congenital CMV infection is 0.4% of births (7). The sociological study of 36 mothers of congenitally CMV-infected children therefore corresponds to a survey of all pregnant women in Malmö over a period of three or four years.

We found no over-representation of nurses among mothers of infants with congenital CMV infection. None of the infants of the nurses had any neurological disorders.

The average age of the nurses with CMV-infected infants was 28 years, as compared with 26 years in the non-nurse mothers with infected infants and 27 years in the control mothers. The number of infants with older siblings was similar in all groups. Primary CMV infection was equally common in the non-nurse and the nurse mothers.

Four studies from the USA or England indicate a low risk of primary CMV infection in nursing personnel (13–16). In Norway, Haneberg *et al.* (17) found a high frequency of CMV among student nurses in paediatric departments, but no further seroconversions occurred after stricter observance of standard

hygienic measures was enforced. Yeager (18) found a trend towards more frequent seroconversion among nurses of infants than among non-nurses. Haldane *et al.* (19) conducted an extensive questionnaire enquiry among nurses in regard to transmissible birth defects. They found that the incidence of congenital defects was significantly higher among the offspring of nurses engaged in the care of infants with congenital defects or of premature infants. The limitations of this enquiry—which were recognized by the authors—included low frequency of replies (54%) and dependence on participants' memories of often distant events. Further, the search comprised the time before rubella vaccination and it did not specifically concern CMV or other stated virus type.

Reports in the literature (13–17) and observations in the present study indicate that when hygienic precautions are satisfactory, infant's nursing attendants are not exposed to heightened risk of contracting CMV. Though the report by Haldane *et al.* (19) is noteworthy, our occupational investigation may be considered more adequate for the local, specific CMV situation. Centralized reporting of specific occupational data during pregnancy (including childminding) would be advantageous for studies of congenital CMV infection.

Exclusion of CMV-seronegative nurses from the care of infants is not practicable. Nor, provided that hygiene is good (no kissing of the infants, hand-washing after changing diapers, etc.) is there clearcut reason why pregnant women should be debarred from such work. It is an open question whether pregnant women exposed to infants with known congenital CMV-infection are at greater risk than those tending infants with undiagnosed congenitally or neonatally acquired infections.

The diagnosis of overt CMV disease during pregnancy presents no problems. Subclinical infections predominate, however, and are less readily diagnosed in the relative absence of indications for serum tests. Preservation of serum sampled as reference prior to concep-

tion from all women who are professionally exposed to CMV is not practicable. As a rule the entire diagnostic procedure must be based on samples obtained postconceptionally. The possibility to recognize fetuses at risk for CMV by tests on maternal sera seems to be limited (4, 20). Obligatory CMV controls of pregnant nurses might do more harm than good.

If there is clear evidence of primary infection early in pregnancy, abortion must be considered. The total risk of transmitting primary maternal CMV infection is about 50% (2, 8) but it might be different in the various trimesters of pregnancy (1, 2, 21). Fetal damage seems to be common when the maternal infection is of primary type and occurs in the first or second trimester (1, 2). Our knowledge on the effect of secondary maternal infection on the offspring is still meagre (3, 4).

For the welfare of the congenitally infected infant, it is essential that normal human contacts be maintained or restored as soon as possible. A congenitally infected infant with only slight CMV excretion should be regarded as normal and handled accordingly.

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IMMUNITY TO CYTOMEGALOVIRUS IN A NORMAL SWEDISH POPULATION  
Comparison with data for pregnant women

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## ABSTRACT

Sera from 1,422 individuals in a normal urban Swedish population representing an age distribution from 1 to 87 years were investigated with the enzyme-linked immunosorbent assay (ELISA) for IgG antibodies to cytomegalovirus (CMV). Forty per cent (27 of 67) in the age group 1-4 years were seropositive. The frequency of seropositives remained fairly constant until the age-groups of 15-19 years in females and 20-24 years in males. From these age-groups on, there was a successive increase. At ages > 40 years, some 85-95 % (544 of 618) of the individuals tested were seropositive. Comparison was made to the antibody status in 4,382 pregnant women, who had been investigated previously. There was a slight overrepresentation of seropositive pregnant women compared with "normal" women in the age-group 15-19 years ( $p < 0.05$ ) but not above this age.

## INTRODUCTION

Human cytomegalovirus (CMV) infection is widely spread all over the world, but the pattern of spread varies both inter- and intranationally. In developing countries, most primary infections occur already in infancy. In one study > 90 % of the children in the age-group 4-8 years were shown to be seropositive, as were 100 % of the pregnant women (10). In Western countries, the acquisition of CMV is generally more delayed (4,7), particularly in high income groups (4). There seem to be two main periods of acquisition, the first in infancy and the second starting in adolescence. The first wave of infection is probably the result of transmission from the mother (1,9), while the second one is a probable effect of intimate contact with other infected individuals. By middle age, 40-90 % of the populations in Western countries have antibodies to CMV (4,7). CMV antibody studies were performed already in 1965 in a Swedish population by Carlström (5). Her studies with the neutralisation test (NT) indicated that more individuals were seropositive than could be detected with the complement fixation (CF) test. The purpose of the present study was to make a new investigation with a more potent antigen introduced since her studies. The investigation was performed using the enzyme-linked immunosorbent assay (ELISA) (11).

## MATERIALS AND METHODS

Sera. The material in the study comprised 1,251 sera from patients (626 males, 625 females) attending the Orthopedic Clinic in Malmö. These patients were considered to represent a normal CMV material. The material had a uniform age distribution from 1-78 years; a few individuals were up to 87 years old. For women in fertile age, the material was extended with sera from 171 female blood donors aged 18-39 years. Results from serological studies on 4,382 pregnant women have previously been reported (2). In all three groups each individual was represented by only one sample. The sera were obtained during the 10-year period 1973-1982 and were kept frozen at  $-20^{\circ}\text{C}$ .

Enzyme-linked immunosorbent assay (ELISA) for demonstration of CMV IgG.

The method used is a modification of that described by Voller et al (11). The modification has been reported in detail previously (2). An alkaline extract of CMV-AD-169 infected human embryonal lung fibroblasts was used as antigen (6). Similarly extracted non-infected cells served as control antigen. The antibody content was expressed as the absorbance ( $E_{405}$ ) difference in

tests performed with serum against virus and control antigens. A difference  $> 0.20$  was considered to indicate a positive reaction.

Statistical analysis. The chi-square test was used to determine the statistical significance of the results.

## RESULTS

The distributions (absolute and percentual) of seropositives in the three materials are listed in Table 1. In the orthopedic material, some 40 % of both males (13 of 32) and females (14 of 35) in the age-group 1-4 years were seropositive to CMV. An increase in the frequency of seropositives started in the age-groups 15-19 years in females and 20-24 years in males and continued until the age of 40 years, after which age some 85-95 % were seropositive in both sexes (males 269 of 311, females 275 of 307).

The frequency of seropositives in the combined materials of female orthopedic patients and female blood donors was 56 % (39 of 70) at ages of 15-19 years, 63 % (45 of 71) at 20-24 years, 71 % (52 of 73) at 25-29 years, 71 % (52 of 73) at 30-34 years and 72 % (57 of 79) at 35-39 years.

For the pregnant women, there was a constant frequency of 71-73 % seropositives in each 5-year period from 15 to 34 years of age; the frequency in the period 35-39 years was 79 %

More pregnant women were seropositive at ages of 15-19 years than were women in the normal material, 128 of 176 and 39 of 70, respectively ( $p < 0.05$ ).

## DISCUSSION

In the present study, about 40 % (27 of 67) of the infants in the orthopedic material were seropositive at ages of 1-4 years, this being independent of sex. The figure is double that reported by Carlström for ages from 7 months to 4 years (22 % or 7 of 32 individuals), but the difference is not statistically significant ( $p > 0.05$ ). Our finding is in good agreement with results from virus isolation studies of infants performed in Malmö (3). The present material is not suitable for observing the eventual disappearance of antibody activity such as has been reported to occur in some cases of CMV infections (1,8). These latter studies were, however, made with the older, less potent CF antigen. Reliable information on antibody disappearance can only be obtained by individual follow-up using a sensitive serologic method. The

question of antibody disappearance is of considerable theoretical interest, since seroconversion later in life could be interpreted falsely as a sign of primary infection.

According to the present serological investigation few new CMV infections occurred between infancy and adolescence.

In the normal female material, a second wave of infections was shown to begin at start of the fertile age, but the percentage of seropositives at this age was still below that of pregnant women. Possible explanations of the higher percentage of seropositive pregnant women in the age-group 15-19 years are overrepresentation of females at the upper age limit, females of lower social and educational status (4) and/or the presence in this group of a larger proportion of immigrants from developing countries (10). Experience has shown that the immigrants become mothers earlier than Swedish women, whose maximum birth-frequency occurs in the age-group 25-29 years (Table 1).

At ages above 40, some 85-95 % of both the men and the women in the normal material were shown to be seropositive. A comparison performed between normal individuals aged  $\geq 50$  years in the present material and in that of Carlström showed that significantly more individuals were seropositive with the ELISA (402 of 446) than with the CF (26 of 41) ( $p < 0.001$ ).

The difference between the present results and those of Carlström is probably due mainly to the less potent antigen used in the latter study. The ELISA technique was not shown to detect significantly more positive reactions than did the CF in a previous study using an equal type of antigen in both tests (2). The general tendency in the present study was in agreement with results from the USA (4).

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Table 1. Immunity to cytomegalovirus in three Swedish populations

| Age<br>(in years)         | No. seropositive / total no. investigated |              |                        |                   |
|---------------------------|-------------------------------------------|--------------|------------------------|-------------------|
|                           | Ortopedic material                        |              | Female<br>blood donors | Pregnant<br>women |
|                           | Males                                     | Females      |                        |                   |
| 1-4                       | 13/32 (41%)                               | 14/35 (40%)  |                        |                   |
| 5-9                       | 24/61 (39%)                               | 30/56 (54%)  |                        |                   |
| 10-14                     | 20/39 (51%)                               | 14/32 (44%)  |                        | 1/1               |
| 15-19                     | 17/39 (44%)                               | 27/42 (64%)  | 12/28 (43%)            | 128/176 (73%)     |
| 20-24                     | 21/31 (68%)                               | 23/34 (68%)  | 22/37 (59%)            | 903/1270 (71%)    |
| 25-29                     | 18/32 (56%)                               | 24/37 (65%)  | 28/36 (78%)            | 1178/1644 (72%)   |
| 30-34                     | 32/42 (76%)                               | 28/38 (74%)  | 24/35 (69%)            | 689/956 (72%)     |
| 35-39                     | 34/39 (87%)                               | 31/44 (70%)  | 26/35 (74%)            | 231/292 (79%)     |
| 40-44                     | 41/49 (84%)                               | 37/45 (82%)  |                        | 32/40 (80%)       |
| 45-49                     | 29/38 (76%)                               | 35/40 (88%)  |                        | 2/3               |
| 50-54                     | 33/41 (80%)                               | 30/35 (86%)  |                        |                   |
| 55-59                     | 32/36 (89%)                               | 28/33 (85%)  |                        |                   |
| 60-64                     | 34/38 (89%)                               | 28/31 (90%)  |                        |                   |
| 65-69                     | 29/30 (97%)                               | 36/36 (100%) |                        |                   |
| 70-74                     | 29/32 (91%)                               | 27/30 (90%)  |                        |                   |
| ≥ 75                      | 42/47 (89%)                               | 54/57 (95%)  |                        |                   |
| Total no.<br>investigated | 626                                       | 625          | 171                    | 4382              |













